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It is a pleasure to write of the life and work of a teacher with whom the writer was so pleasantly associated in both teaching and investigation for sixteen years. Professor Conn takes his place among the prominent biologists of the nineteenth century. His achievements are particularly interesting and noteworthy because they cover the pioneer period of the branches of science which he made his specialties. He was at the front in the great advance made in biological science in the past thirty years, but at no time lost his deep interest in the social welfare of all mankind. His dominant work was in dairy bacteriology, although he taught and expressed some of the most profound principles of agricultural science in the relation of bacteria to crop production. He was always on the right side of all public and social problems, although he was given ample opportunities to testify on the questionable side of public welfare controversies at large remuneration.

As a teacher, perhaps, he reached his highest achievement, as is attested to by more than two thousand students who came under his influence. His personal magnetism, polished English, interesting illustrations and illuminating suggestions captivated his listeners. His teaching was masterful, and with him it became an art. He was able to make a difficult subject easily understood, and the breadth of his conception of the subject at hand was remarkable. This breadth of view acquired from very extensive reading made a lasting impression on the mind of the student. No one could attend his lectures without acquiring a wider and truer view of life and its problems. In laboratory teaching his pleasant suggestiveness and persuasive leading stimulated the student to acquire knowledge for the mere pleas-

sure of it. His was the method of Agassiz. He instructed his assistants to give as little help as possible until the student had reached the limit of his own observation. "Let them find it out for themselves," was the trite saying which proved that he well knew the true methods of acquiring an education. He was able by clear and concise directions to conduct laboratory sections in general biology for over seventy students at a time.

As a member of society he could converse entertainingly and instructively on almost any topic of interest. He had the courage to attack any problem, no matter how difficult, and always labored at its solution with great perseverance.

The most interesting phase of his life's work was accomplished in dairy bacteriology. At the time of the founding of the Storrs Agricultural Experiment Station by Dr. Atwater in 1889, Dr. Conn commenced the studies of the organisms which ferment milk. In the first report of this Station will be found an account of this early work. In 1890 he isolated bacterial enzymes from pure culture which when added to milk in powder form caused a curdling and subsequent digestion. In the fall of 1891 was commenced the study of the organisms that ferment ripening cream. As each kind of bacteria was isolated it was given a number. When the exhibit was installed at the World's Fair, Chicago, in 1893, to show the effect of bacteria in the production of flavor in butter, some thirty-five varieties of organisms had been isolated. No. 2 at that time produced the best flavor, and No. 16 the worst. The latter was sickening, brackish, and persistent. These two organisms were used daily at the fair in the ripening of cream for churning. An upright case was installed in the exhibit. This case held about forty large test tubes which showed the effect of pure cultures growing in milk. The exhibit attracted a great deal of attention and the city papers published long descriptions of it. During the same summer eight or nine varieties of bacteria were added at the fair to the list, most of them isolated from a can of milk from Uruguay. Among them was the remarkable and famous "B 41." Following this came the development and application of cream starters which gained practically universal use throughout the

United States. At the same time blue litmus gelatin was developed for the isolation of acid bacteria. From this experimental work in the bacteriology of milk has been built up a fairly extensive science of dairy bacteriology.

As director for several years in the Summer School at Cold Spring Harbor, Long Island, his capabilities reached their highest achievement. The writer's memories of him at this place are the most cherished of all. We were a happy family. All restraint of forced attendance was removed, but we could not afford to miss a single exercise. His lectures here, as elsewhere, were models of presentation and scope. As there was no church in the immediate vicinity, Dr. Conn would address us Sundays on some appropriate topic.

In his interest of scientific agriculture for the welfare of mankind, and with keen perception and prophetic vision, he presented the problems many years ago which we are today trying to solve. In 1882 Atwater and Woods demonstrated that the legumes were capable of fixing definite amounts of atmospheric nitrogen. Nine years later Professor Conn explained the function of bacteria in the fixing of this nitrogen in the root nodules of the legumes. To the writer a solution of these problems for the benefit of the world appears to be a most worthy goal.

Dr. Conn's influence as a Christian gentleman who was always an exponent of right, teaching the profound philosophy of correct living, will long make itself felt among his many students, associates and friends.

THE FAMILIES AND GENERA OF THE BACTERIA

PRELIMINARY REPORT OF THE COMMITTEE OF THE SOCIETY OF AMERICAN BACTERIOLOGISTS ON CHARACTERIZATION AND CLASSIFICATION OF BACTERIAL TYPES

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In the last presidential address before the Botanical Society of America, Dr. A. S. Hitchcock presented an interesting comparison between the dominant position of taxonomy in the botany of the eighteenth and early nineteenth centuries and its relative neglect today, and made a strong plea for the recognition of the very real importance of this branch of botanical science. "To me," he says, "the two great questions that botanists seek to answer are, "*How do plants live?*" and "*How are plants related?*" Most botanical investigations can be used as an aid in answering one or the other of these questions. From this standpoint the two fundamental divisions of botany are physiology and taxonomy." (Hitchcock, 1916.)

Botanists engaged in the study of the higher plants may perhaps neglect systematic problems with a certain degree of impunity, because so many of the more fundamental questions involved have been settled by the workers of an earlier period. Bacteriology however has never passed through a taxonomic phase; and it is unnecessary to rehearse at length our sound reasons for dissatisfaction with the present crude state of bacterial classification. It is obvious to all students of this group that the characterization of specific types is generally obscure and that the classification of these types into larger generic and family groups is almost ludicrously artificial.

With the first of these problems, the characterization of bacterial species, we are not at present concerned. The Society of American Bacteriologists at its Urbana meeting specifically

directed its Committee on Bacterial Classification to prepare an outline of classification of the Bacteria into families and genera. It is this task which we have attempted, with full recognition of the impossibility of reaching a final result, but in the belief that our present state of knowledge makes possible material advances over the classifications now in common use. We have been all the more ready to undertake the work because we have felt that if it were not undertaken by bacteriologists it would probably be attempted by botanists, unfamiliar with the peculiar characteristics of the organisms concerned.

I. PREVIOUS SYSTEMS OF BACTERIAL CLASSIFICATION

The present chaotic condition of bacterial classification is in large measure due to the carrying over into bacteriology of the conception—valid enough among the higher plants—that classification must be based primarily on visible structure or morphology. It has been pointed out in a presidential address before the Society that physiological characters are quite as suitable for systematic uses as structural differences.

> There is no fundamental distinction between morphological and physiological properties, since all are at bottom due to chemical differences in germ plasm, whether they happen to manifest themselves in the size and arrangement of parts or in the ability to utilize a certain food stuff. Indeed biochemical properties have a peculiar and unique significance among the bacteria, since it is precisely along the lines of metabolism that these organisms have attained their most remarkable differentiation. The higher plants and animals have developed complex structural modifications to enable them to obtain food materials of certain limited kinds. On the other hand the bacteria have maintained themselves by acquiring the power of assimilating simple and abundant foods of varied sorts. Evolution has developed gross structure in one case without altering metabolism; it has produced a diverse metabolism in the other case, without altering gross structure. There is as wide a difference in metabolism between the pneumococci and the nitrifying bacteria as there is in structure between a liverwort and an oak. (Winslow, 1914.)

Yet the only bacterial genera named and described up to 1896, and the ones which are still in general use today are based solely on morphological or structural characters, some of them of no significance, whatever, while physiological differences of the most fundamental importance are ignored.

The foundation for the current classification of bacteria was laid by Ehrenberg (1838) nearly a century ago. He divided his Vibrionia, which corresponded to the Bacteria, into five genera: *Bacterium* (straight, rigid filaments); *Vibrio* (straight filaments, showing serpentine motion); *Spirillum* (rigid spiral filaments); *Spirochaeta* (flexible spiral filaments); and *Spirodiscus* (spirals flattened, disc-like). Hoffmann (1869) recognized that the spherical forms previously treated separately must be classified with the other bacteria under Hallier's name of *Micrococcus*. Ferdinand Cohn in 1872 laid special stress on the grouping of the bacterial cells in chains or in zooglea masses. He distinguished four main groups (Tribus): Sphaerobacteria, spherical or oval, non-motile cells, often in zooglea masses (*Micrococcus*); Microbacteria, short cylindrical cells, motile, often in zooglea masses, (*Bacterium*); Desmobacteria, growing in threads without zooglea (*Bacillus, Vibrio*); Spirobacteria, motile spirals, without zooglea (*Spirochaeta, Spirillum*). Three years later Cohn proposed another classification in which these and other genera of the bacteria were separated from each other and grouped respectively with supposedly similar forms belonging to the blue-green Algae.

Perhaps the most significant bacterial classification after that of Ehrenberg was the system proposed by Zopf (1883–5). He retained a distinct group for the spherical bacteria (Coccaceae), reunited the rods and the spirals (Bacteriaceae); and introduced the groups Leptothriceae and Cladothriceae for the filamentous forms. His genera are tabulated below. The most important advance in this classification was the differentiation between the spore-forming organisms (*Bacillus*) and the non-spore-formers (*Bacterium*).

ZOPF'S CLASSIFICATION

I. Coccaceae

(Spherical elements, no spores, division in one or several planes)

1. *Streptococcus.* (Chains)
2. *Merismopedia.* (Plates)
3. *Sarcina.* (Packets)
4. *Micrococcus.* (Irregular masses)
5. *Ascococcus.* (Similar to Micrococcus, but showing marked zooglea formation)

II. Bacteriaceae

(Cocci, straight or curved rods and straight or spiral filaments, latter never showing differentiation between base and apex, division in one plane)

1. *Bacterium.* (Cocci and rods without spores)
2. *Spirillum.* (Spirals without spores)
3. *Vibrio.* (Spirals with spores)
4. *Leuconostoc.* (Cocci and rods, former showing spore formation)
5. *Bacillus.* (Cocci and rods with spores)
6. *Clostridium.* (Like *Bacillus,* but spores in spindle-shaped elements)

III. Leptothriceae

(Cocci or rods, threads or spirals, threads showing differentiation between base and apex, no spores.)

1. *Crenothrix.* (Threads with sheath, cells without sulphur, water forms)
2. *Beggiatoa.* (Threads without sheath, cells with sulphur granules, water forms)
3. *Phragmidiothrix.* (Cells without sheath, segmentation pronounced, cells without sulphur, water forms)
4. *Leptothrix.* (Threads, with or without sheath, segmentation not pronounced, cells without sulphur)

IV. Cladothriceae

(Cocci, rods, threads or spirals, threads showing false branching. No spores)

1 *Cladothrix*

Van Tieghem and de Bary laid even more stress on spore formation, dividing the bacteria sharply into an endosporous and an arthrosporous series. Hueppe followed Zopf's classification quite closely, introducing the genera *Staphylococcus* and *Leuconostoc* among the Coccaceae, separating the spiral form under the group-heading Spirobacteriaceae, and rearranging the genera under this head as follows:

Spirobacteriaceae

1. *Spirochaeta.* (Without endospores)
2. *Spirillum.* (With endospores)
3. *Vibrio.* (With endospores, and change of form in spore formation)

The same general plan of classification, based on cell form and spore production, was carried a step further by Lehmann and Neumann in 1896. The following outline is from the 1904 edition of their work.

I. Coccaceae

1. *Streptococcus.* (Dividing in one plane)
2. *Sarcina.* (Dividing in three planes)
3. *Micrococcus.* (Irregular division, including all but clearly marked chains and packets)

II. Bacteriaceae

1. *Bacterium.* (Without endogenous spores, rods usually 0.8–1.0μ in diameter)
2. *Bacillus.* (With endogenous spores, rods often more than 1.0μ in diameter)

III. Spirillaceae

1. *Vibrio.* (Short, rigid, slightly curved cells, with 1 to 2 polar flagella)

2. *Spirillum.* (Long rigid spiral cells, with lophotrichic flagella)
3. *Spirochaeta.* (Long flexible spiral cells, flagella unknown, motility accomplished by an undulating membrane)

Supplementary Group

ACTINOMYCETES

Thread-like cells with true branching, sometimes even a richly branching mycelium. Young cultures often show only normal unbranched bacterial cells. Many forms show a tendency to the formation of irregular clubbed cells.

1. *Corynebacterium.* (Slender, often slightly curved rods, often with a tendency to club formation, branching rare in young cultures and often difficult to find even in old ones. Neither flagella nor spores known. With weak stains show barred staining. Not acid-fast)
2. *Mycobacterium.* (Same as above except for the fact that the cells are acid-fast and take ordinary stains with difficulty, and that club forms are very rare outside the body)
3. *Actinomyces.* (Long, mycelial threads, showing true branching. Spores formed by fragmentation and cross division of filaments. Many forms produce a mold-like aerial mycelium. Not acid-fast. Motility sometimes present. Most forms produce a moldy odor)

This outline of Lehmann and Neumann's seems to us the most satisfactory classification that could be worked out on purely morphological grounds. In 1894, however, Migula suggested a new system in which motilty was used instead of spore formation as a secondary basis of classification. The outline below is from his later work, System der Bakterien (1900).

MIGULA'S CLASSIFICATION

Order *Eubacteria*

Cells without nuclei, sulphur or bacteriopurpurin, colorless or but slightly colored, some forms chlorophyll-green.

Family I. Coccaceae

Cells when free spherical, in division sometimes elliptical. Division in one, two or three planes of space without previous elongation of the cell. Motility and endospore formation rare.

1. *Streptococcus.* (Spherical cells dividing in one plane, often remaining attached to each other to form pairs or chains. Zooglea-like sheath or capsule common. Non-motile. No endospores)
2. *Micrococcus.* (Division in two planes, sometimes forming bands of cells. Non-motile, endospores probably absent)
3. *Sarcina.* (Division in three planes at right angles to each other, forming packets when the cells remain attached to each other. Non-motile. Endospore formation doubtful)
4. *Planococcus.* (Division in two planes. Flagella present, usually 1 to 2 in number. No endospores known)
5. *Planosarcina.* (Division in three planes, pairs and tetrads usually seen rather than packets. Flagella present, usually one to each cell. No endospores)

Family II. Bacteriaceae

Cells cylindrical rods in free condition, dividing in a plane at right angles to their length. Short-celled forms may be distinguished from cocci by elongation before division. Cells may remain united forming long or short threads. No sheath.

1. *Bacterium.* (No flagella. Some form spores, others do not)

2. *Bacillus.* (Peritrichic flagella. Spore formation common)
3. *Pseudomonas.* (Polar flagella, 1 to 10 in number. Endospores in some forms)

Family III. Spirillaceae

Cells more or less spirally curved, division in one plane at right angles to long axis. Spore formation rare. Most forms motile, with polar flagella.

1. *Spirosoma.* (Broad rigid cells, non-motile, free or in small zooglea groups)
2. *Microspira.* (Cells usually comma- or sausage-shaped, motile with one or rarely 2 to 3 polar flagella. Endospores not observed)
3. *Spirillum.* (Long or short spirals, lophotrichic flagella, spores sometimes observed)
4. *Spirochaeta.* (Slender spiral cells, generally long and flexible, showing serpentine and screw-like motions. Organs of motility unknown. Spores not observed)

Family IV. Chlamydobacteriaceae

Cylindrical cells arranged in threads with sheath. Reproduction by conidia which arise directly from the vegetative cells and develop into new threads without any resting stage.

1. *Chlamydothrix.* (Unbranched filaments, segments often demonstrable by the use of reagents. Conidia non-motile)
2. *Crenothrix.* (Unbranched filaments with differentiation between base and apex. Sheath thick and in iron waters often permeated with hydrate of iron. Cells cylindric or flat discoidal, conidia non-motile)
3. *Phragmidiothrix.* (Very long threads with delicate sheath. Conidia non-motile)
4. *Sphaerotilus.* (Cells in dichotomously branched threads, conidia motile)

Order *Thiobacteria*

Cells with no nucleus but with inclusions of sulphur, colorless or colored red, rose or violet with bacteriopurpurin. Never green.

This order includes the families Beggiatoaceae (genera *Thiothrix* and *Beggiatoa*) Rhodobacteriaceae (genera *Thiocystis, Thiocapsa, Thiosarcina, Lamproystis, Thiopedia, Amoebobacter, Thiothece, Thiodictyon, Thiopolycoccus chromatium, Rhabdochromatium* and *Thiospirillum*) into the classification of which we need not enter here.

An even more elaborate morphological classification of the bacteria is that suggested by A. Fischer in 1895, and later elaborated and altered in successive editions of his Vorlesungen über Bakterien. The outline below is taken from Coppen Jones' translation of the latter work (Fischer, 1900).

Order I. *Haplobacterinae*

Vegetative phase unicellular, spherical, cylindrical, or spirally twisted; isolated or united in chains or clusters.

Family I. Coccaceae

Vegetative cell spherical.

Sub-family I. *Allococcaceae*

Planes of fission without definite sequence; no pronounced colonies or growth-forms, cells isolated or in short chains or irregular clusters.

Genus *Micrococcus* Cohn. Non-motile. Includes most cocci, the pathological 'staphylococci,' etc.

Genus *Planococcus* Migula. Motile.

Sub-family 2. *Homococcaceae*

Planes of fission in definite sequence.

Genus *Sarcina* Goodsir. Three planes of division at right angles to each other. Cubical colonies, non-motile.

Genus *Planosarcina* Migula. Similar to *Sarcina*, but monotrichous, ciliate, and motile.

Genus *Pediococcus* Lindner. Two planes of fission, alternate and at right angles. *Micrococcus tetragenus Thiopedia* (a sulphur organism), and probably some species usually termed Micrococcus.

Genus *Streptococcus* Billroth. Planes of fission parallel, giving rise to chains; the pathological *Streptococci* and *Leuconostoc.*

Family 2. Bacillaceae

Vegetative cell straight, cylindrical, ellipsoidal or egg-shaped; very short forms difficult to distinguish from cocci. Fission always transverse.

Sub-family I. *Bacilleae*

Spore-forming rods cylindrical, unchanged.

Genus *Bacillus* Cohn. Non-motile. *B. anthracis*, *B. diphtheriae*, etc.

Genus *Bactrinium* A. Fischer. Motile, monotrichous, with terminal cilium: includes provisionally all monotrichous rods whose spores are as yet unknown, e.g., *B. pyocyaneus.*

Genus *Bactrillum* A. Fischer. Motile, with lophotrichous ciliation. Includes provisionally *B. cyanogenus*, and many other sporeless forms.

Genus *Bactridium* A. Fischer. Motile, peritrichous, in some spores as yet unknown. Very numerous representatives, e.g. *B. subtilis*, *B. megatherium*, *B. vulgaris* (old genus Proteus), *B. typhi*, and *B. coli.*

Sub-family 2. *Clostridieae*

Rods spindle-shaped during sporulation.

Genus *Clostridium* Prazmowski. Motile, peritrichous; includes some of the butyric bacteria. Genera with monotrichous and lophotrichous ciliation are unknown as yet.

Sub-family 3. *Plectridieae*

Rods drumstick-shaped during sporulation.

Genus *Plectridium* A. Fischer. Motile, peritrichous; some butyric bacteria, the organism causing tetanus, and a methane fermenter.

Family 3. Spirillaceae

Vegetative cell cylindrical, but spirally twisted. Fission always transverse.

Genus *Vibrio* Miller and Löffler. Very slightly curved rods, 'comma'-shaped; motile, monotrichous. *Vibrio cholerae asiaticae* and numerous other vibrios of fresh and salt water.

Genus *Spirillum* Ehrenberg. Cylindrical cells twisted in an open spiral; motile, lophotrichous. *Spirillum undula, Sp. rubrum.*

Genus *Spirochaeta* Ehrenberg. Cells long and attenuated, spirally twisted with numerous turns; cilia unknown; the cell membrane is perhaps yielding. *Spirochaeta Obermeieri* (remittent fever).

Order 2. *Trichobacterinae*

Vegetative phase an unbranched or branched filament or chain of cells, the individual members of which break off as swarm-spores (*gonidia*).

Family I. Trichobacteriaceae

(a) Filaments non-motile, rigid, enclosed in a sheath.

Genus *Crenothrix* Cohn. Filaments unbranched and devoid of sulphur granules.

Genus *Thiothrix* Winogradsky. The same, but containing sulphur granules.

Genus *Cladothrix* Cohn. Filaments branched, false dichotomy (includes *Sphaerotilus*).

(b) Filaments motile, with oscillating and gliding movements, and devoid of a sheath.

Genus *Beggiatoa* Trevisan. Containing sulphur.

The prominent place given to motility seems to us to constitute a peculiar infelicity in these schemes. Spore formation marks off at least two fairly distinct natural groups of bacteria; but motility is a character of singularly slight taxonomic importance among the bacteria.

> No one familiar with the colon group can hold that it is reasonable to place the common type of motile colon bacillus in the genus *Bacillus* along with *B. mycoides*, *B. aerogenes*, *B. anthracis*, *B. prodigiosus*, *B. radicicola* and *B. tetani*, and to place an organism having all its other properties identical but lacking flagella in the genus *Bacterium*. The same arguments hold true against the genera *Planococcus* and *Planosarcina* among the cocci. We find in several of the major groups motile and non-motile forms which are precisely alike in half a dozen respects and are clearly minor varieties of the same type, and it is absurd to give them generic rank, and group together widely different types which are alike in no single respect except that they have flagella. These genera based on motility are on a par with a division of animals into those with wings and those without, which would place bats and birds and flying fishes and bees in one group, and cats and ordinary fishes and worker ants in another. (Winslow, 1914.)

Unfortunately, however, it is precisely Migula's defective classification which has for some reason established itself in the literature of American bacteriology. Chester (1901) accepted it *in toto*, with the addition of Lehmann and Neumann's family *Actinomycetes*, which he called *Mycobacteriaceae*, and in which he included two genera only, *Mycobacterium* and *Streptothrix*. Erwin Smith (1905) also adopted the view that genera should be fixed by morphological characters only and accepted Migula's genera with the change of three names. He substituted *Vibrio* Miller for *Microspira* Schroeter; *Bacterium* Cohn for *Pseudomonas* Migula; and Aplanobacter (nov. gen.) for *Bacterium* Ehrenberg.

The classifications of Zopf and Lehmann and Neumann seem to us to be very much more satisfactory than those of Migula and Fischer. Like all the earlier workers in bacteriology, however, these authors left the great mass of the rod-shaped bacteria, including hundreds of enormously varied types, in the two gen-

era, *Bacterium* (small non-spore-formers) and *Bacillus* (large spore-formers).

It is to W. Kruse (1896) that we owe the first recognition that these unwieldy and heterogeneous genera must be broken up by taking into account the physiological as well as the morphological characters of the organisms concerned.

Kruse enumerates fifteen different sorts of characters by which the bacteria may be classified, giving first place to morphological differences, such as the spore formation, the presence of a gelatinous envelope, and motility, but recognizing also the importance of staining reactions, ability to develop in certain culture media, relation to temperature and oxygen, biochemical powers, pigment production, pathogenicity and character of growth in media. It is true that he considers the physiological characters as of minor and doubtful value and so variable as not to warrant giving the groups of bacteria definite generic names. Yet he presents at the end of his discussion the following list of numbered groups which represents by far the most valuable plan of classification suggested up to 1909. There will be little question of the fact that most of the types listed represent natural groups easily recognizable by all working bacteriologists. In the characterizations below Kruse's summary table (pp. 93–95) has been in some cases supplemented by data given in his text.

KRUSE'S CLASSIFICATION

I. Coccaceae

A. *Streptococcus.* Division in one plane

1. Saprophytic streptococci, usually short, often liquefying gelatin, sometimes showing marked zooglea formation (*Leuconostoc*).
2. Parasitic streptococci, usually long
   a. *Diplococcus pneumoniae* type
   b. *Streptococcus pyogenes* type

B. *Merista.* Division in two planes at right angles to each other.

1. Tetragenic group. Typical arrangement of cells in tetrads

2. *B. gonorrhoeae* group. Cells usually in pairs. Gram-negative
3. *Staphylococcus pyogenes* group. Development in two planes often interrupted, giving pairs, tetrads and also short chains

C. *Sarcina*. Development in three planes at right angles to each other.

II. Bacillaceae

1. Group of the colorless sulphur bacteria; (*Beggiatoa, Thiothrix*) usually large unbranched threads, without spores, utilizing sulphur in their metabolism
2. *Leptothrix* group. Non-cultivable water organisms, sulphur-free, unbranched, which form large threads without spores
3. *Cladothrix* group. Usually non-cultivable water organisms which form large threads and show false branching. (Spores formed in *Cladothrix intricata* which can also be cultivated in nutrient media)
4. Hay bacillus group. Usually large bacilli, commonly isolated or in small groups, when in long chains never showing base and apex differentiation as in many forms of the first three groups. Spores formed without change in the shape of the mother cell. Gram-positive. Saprophytes. Easily cultivable. Generally liquefy gelatin. Spore germination probably equatorial
5. Anthrax group. Distinguished from preceding group only by fact that spore germination is probably polar
6. Malignant Oedema group. Large spore-bearing anaerobic bacilli. Saprophytic or parasitic. Colonies on agar usually stellate. No change in form of mother cell on spore formation. Less easily stained by Gram method than preceding group. Usually liquefy gelatin and produce foul odors

7. Symptomatic Anthrax and Butyric Acid group. Differs from preceding group in forming clostridium spores
8. Tetanus group. Differs from preceding groups in forming drumstick spores
9. Proteus group. Aerobes or facultative anaerobes of medium size decolorized or irregularly stained by Gram. No spores. Colonies resemble those of preceding groups in showing amoeboid or stellate outgrowths sometimes separating as daughter colonies; also in producing malodorous decomposition of protein. Cells vary from coccoid forms to long, sometimes twisted filaments

   Supplementary group: liquefying, pathogenic forms
10. Fluorescent group. Non-spore-bearing bacilli, generally of medium size. Gram-negative
11. Pigment-forming group. A somewhat artificial group including red, brown, yellow, blue, and violet chromogens
12. Water Bacilli. Easily cultivable saprophytic bacilli of medium size, non-spore-forming, Gram-negative, usually motile and gelatin-liquefying. (Root nodule bacteria and plant pathogens included)

    Supplementary group: marine phosphorescent forms, often curved rods
13. Nitrobacteria. *Nitrosomonas* and *Nitrobacter* types. (Nitrogen fixers of the soil also included)
14. Aerogenes and Rhinoscleroma group. Non-motile, non-spore-bearing, non-liquefying, Gram-negative bacilli of medium size, usually plump. Easily cultivable. Facultative anaerobes. Tendency to capsule formation. Surface colonies on gelatin drop-like

    Supplementary group: Acetic and lactic acid bacteria, and bacteria of cheese and slimy fermentation

15. Colon-Typhoid group. Distinguished from preceding group by motility, and with less constancy by lack of capsules, more expanded surface colonies on gelatin and tendency for rods to be longer and more slender
16. Haemorrhagic Septicemia group. Distinguished from preceding group by high pathogenicity. Motile or non-motile

Supplementary group. Bacilli of human haemorrhagic infection (includes *B. pestis*)

17. B. tenuis sputigenus group. Gram-positive, non-spore-bearing, non-liquefying, bacilli of varying size. Motile or non-motile. Facultative anaerobes
18. Influenza group. Very small isolated bacilli, Gram-negative, non-spore-bearing, difficult of cultivation, most forms obligate parasites
19. *Swine erysipelas* group. Differing from preceding group by Gram-positive staining. Moderate growth on media. Highly pathogenic
20. Glanders and Pseudotuberculosis group. Small bacilli isolated or in chains, non-spore-forming, usually Gram-negative, growing poorly on media. Specially characterized by slow metastatic growth in tissues and production of granulating centers
21. Diphtheria group. Small or medium-sized bacilli, swollen and clubbed in older cultures. Non-spore-bearing, Gram-positive, non-liquefying. Obligate parasites. Pathogenic forms either develop locally producing a powerful toxin or produce metastases as in preceding group. Dividing cells sometimes slip round into parallel position forming palisade grouping. Barred and granular staining in old cultures. Branched cells occasionally observed
22. Tuberculosis group. Small, slender, non-motile bacilli, Gram-positive, highly resistant to ordinary stains. Probably non-spore-forming, obligate parasites, growing very slowly on media. Metastatic

development in body. Tendency to irregular staining. Club forms and branched forms occur

III. SPIRILLACEAE

1. Saprophytes, cultivated with difficulty or not at all
2. Saprophytes, cultivable, non-liquefying
3. Saprophytes and parasites, easily cultivable, liquefying
4. Obligate parasites

Kruse's treatment of the spherical and spiral forms is obviously very casual (as he admits in a footnote). His groups of the bacilli however show wide knowledge and clear analysis. With our present information we can combine some of his groups and subdivide others; but most of them represent clear and definite entities, and the outline above deserves the serious consideration of anyone engaged in the task of systematic bacteriology.

The next contribution to the broader questions of taxonomy was the work of the Winslows (1908) on the spherical bacteria. They emphasized two points in particular, the value of the frequency curve in fixing specific types and the importance of correlations of characters in defining generic groups.

The application of the second principle led these authors in the first place to recognize two distinct subfamilies within the *Coccaceae*. The cocci of the first group, comprising most of the forms from the body, showed as a rule chains and irregular cell-grouping, stained by the Gram method, gave a meager or only fair growth on media, formed acid from carbohydrates and produced no pigment or a white or orange one. The cocci of the other group, for the most part from soil or water, often showed packet formation, were usually Gram-negative, grew well on media, failed to ferment carbohydrates and produced a yellow or red pigment. "Each character was occasionally found in the group where it did not usually occur; but the correlation of properties in the vast majority of cases was very strong."

The definitions of subfamilies and genera were given as below:

WINSLOWS' CLASSIFICATION

COCCACEAE

A. Subfamily *Paracoccaceae*

Parasites. Growth not abundant (or, one species, zoogloea-forming saprophytes, yielding abundant growth in saccharose media). Generally Gram-positive. Acid formers.

1. *Diplococcus.* Cells in capsulated pairs. Parasites. Growth very meager. Inulin fermented. No pigment
2. *Ascococcus.* Cells in chains occurring in masses of zooglea in sugar refineries. Aberrant saprophytic form. Growth abundant in saccharose media. No pigment
3. *Streptococcus.* Cells in chains. Parasites. Growth meager. Inulin not fermented. No pigment
4. *Aurococcus.* Cells in irregular groups. Parasite. Growth fair. Orange pigment
5. *Albococcus.* Cells in irregular groups or in fours. Parasites. Growth good. White pigment

B. Sub-family *Metacoccaceae*

Saprophytes. Growth abundant. No zooglea. Generally Gram-negative. Not acid formers.

1. *Micrococcus.* Cells in irregular groups. Pigment generally yellow
2. *Sarcina.* Cells in packets. Pigment yellow
3. *Rhodococcus.* Cells in irregular groups or packets. Pigment red

It appeared from this study that the packet grouping to which so much importance had previously been attributed was correlated with no other significant character, the genera *Micrococcus* and *Sarcina* being absolutely parallel to each other in all other respects. On the other hand, pigment formation which had been so lightly thought of that the orange, yellow and white staphylococci were treated as varieties of a single species, was markedly correlated with important physiological differences.

Thus the white and orange chromogens were usually parasitic in origin, Gram-negative, and non-fermenters, while the yellow and red forms differed in all these respects. Even the closely related white and orange groups, while both included liquefiers and non-liquefiers, showed a perfectly characteristic difference in the amount of liquefaction produced by the liquefying forms, the white cocci liquefying slowly and the orange cocci very rapidly. The red cocci showed feeble liquefaction and vigorous nitrate reduction.

Buchanan (1915) has justly questioned the validity of the generic terms applied to their groups by the Winslows, and has pointed out that many of the names should be modified to conform to the International Rules for Botanical Nomenclature.

There remains to be considered but one other system of bacterial classification, the revolutionary and illuminating monograph of Orla Jensen (1909).

The chief contention of this author concerns the supreme importance of physiological characters, which he maintains should furnish a primary basis of classification among the bacteria just as chemical composition is more fundamental than crystalline form in the classification of minerals. He points out the significant fact that the three major morphological types of the ordinary bacteria are paralleled in the clearly homogeneous group of the red sulphur bacteria.

The presence or absence of flagella Jensen rightly considers as of very minor importance. The arrangement of the flagella, when they are present, happens on the other hand to be correlated with fundamental physiological differences, and the author gives the two orders into which he divides the bacteria the names of *Cephalotrichinae* (monotrichous or lophotrichous) and Peritrichinae (peritrichous). The *Cephalotrichinae*, deriving their life energy almost entirely from oxidative processes, are all water or moist earth forms, with the exception of a few peculiar plant and animal parasites, and for the most part grow badly or not at all on ordinary organic media, and spores are never formed. The second order, the *Peritrichinae*, includes the more specialized bacteria in whose metabolism the splitting of carbohydrates or amino-acids plays a primary rôle rather

than oxidation or denitrification. They are rods or cocci, peritrichous when possessing flagella at all, and among them are found all the commoner putrefactive and parasitic types.

In general the bacteria which derive their life energy from inorganic sources are clearly most primitive, while Jensen considers those forms which derive their energy from carbohydrates and their nitrogen from inorganic nitrogen compounds as coming next, the types requiring nitrogen in organic form being the highest. He points out that the opportunity to utilize such substances as milk sugar and urea must necessarily have come only in relatively recent geologic time, and that human pathogens must be among the youngest of bacterial types.

Resting in the main on a detailed consideration of those metabolic processes, Jensen divides the *Cephalotrichinae* into seven and the *Peritrichinae* into four families. The first series begins with the Oxydobacteriaceae, including the most primitive bacteria, which oxidize methane and carbon monoxid, the nitrifiers, the acetic acid bacteria and the Azotobacter group. Then follows the Actinomyces family which includes the root nodule bacteria and the mycobacterium (tuberculosis) group. The collocation of the latter forms is startling at first, but their morphology, their oxygen requirements and their unique pathological relations, almost symbiotic by contrast with the quick toxic action of other pathogenic bacteria, offer some evidence of real relationship. The third, fourth and fifth families are the Thiobacteriaceae (the sulphur bacteria), the Rhodobacteriaceae (the red or purple sulphur bacteria) and the Trichobacteriaceae (*Cladothrix*, *Crenothrix*, *Beggiatoa*, etc.) which are clearly natural groups. The last two families, the Luminibacteriaceae and the Reducibacteriaceae, are typically denitrifying organisms which form a connecting link between the primitive oxidizing bacteria and the *Peritrichinae*. They include the fluorescent water bacteria and the phosphorescent vibrios and at the higher end of the series such types as the cholera organism in which the ability to split complex products with the formation of lactic acid and indol begins to appear.

The second order, the *Peritrichinae*, is divided into four fami-

lies. The first, the Acidobacteriaceae, includes the non-spore-forming carbohydrate fermenting types, among the principal representatives being the colon-typhoid group and practically all the cocci. His second family, the Alkalibacteriaceae, shows a higher development of the power of decomposing nitrogenous bodies, and includes the liquefying proteus forms, the actively liquefying aerobic spore formers and certain urea fermenters. The last two families, the Butyribacteriaceae and the Putribacteriaceae, are made up of the strict anaerobes.

The broad outlines of this classification are highly suggestive, and the emphasis on biochemical properties is a long step in the right direction. When one studies the families and genera in detail, however, the result is somewhat disappointing. Professor Jensen rides his physiological principle much harder than even Migula did his morphological one, and makes no attempt at a judicial consideration of the grouping of common characters which should mark natural biological subdivisions. His disregard of the rudimentary principles of terminology is absolute. He coins new names for well established groups with entire freedom, according to the simple principle that the names of rod forms with polar flagella should end in *monas*, or rod forms with peritrichic flagella in *bacterium*, or spore-bearing rods in *bacillus*, etc., and with these suffixes he compounds roots expressing the chemical activity of the organisms concerned. Thus for example he changes *Azotobacter* to *Azotomonas* and *Mycobacterium* to *Mycomonas*, and upsets almost all the generic names of the red sulphur bacteria in pursuance of this plan.

Finally, Jensen nowhere gives clean-cut definition of any of his groups, and it is often difficult or impossible to draw any clear distinction between them. The outline below represents the most complete characterization we have been able to derive from a careful study of the text.

JENSEN'S CLASSIFICATION

A. Order *Cephalotrichinae*

Monotrichic or lophotrichic when flagella are present. Life energy derived mainly from oxidative processes without produc-

ing notable amounts of unoxidized split products. Water or moist earth forms, with the exception of a few peculiar plant and animal parasites. Grow poorly or not at all on artificial media. No spores formed.

1. Family Oxydobacteriaceae

Obligate aerobes, deriving their energy from direct oxidation of simple compounds of carbon, hydrogen and nitrogen. Mostly short rods, unbranched and monotrichic, except genera 4 and 7.

1. *Methanomonas.* Oxidizing methane to carbon dioxid and hydrogen
2. *Carboxydomonas.* Oxidizing carbon monoxid to carbon dioxid
3. *Hydrogenomonas.* Oxidizing hydrogen to water
4. *Acetimonas.* Oxidizing alcohol to acetic acid, form surface films
5. *Nitrosomonas.* Oxidizing ammonia
6. *Nitromonas.* Oxidizing nitrites
7. *Azotomonas.* Nitrogen fixers, branched cells occur

II. Family Actinomycetes

1. *Rhizomonas.* (*Rhizobium*, symbiotic in nodules of legumes, Lehmann and Neumann)
2. *Corynemonas.* (*Corynebacterium*, L. & N.)
3. *Mycomonas.* (*Mycobacterium*, L. & N.)
4. *Actinomyces.* (L. & N.)

III. Family Thiobacteriaceae

Colorless sulphur bacteria. Non-filamentous.

1. *Sulfomonas.* Rods, oxidize hydrogen sulfide only to sulphur
2. *Thiomonas.* Rods
3. *Thiococcus.* Cocci
4. *Thiospirillum.* Spirals

IV. Family Rhodobacteriaceae

Red sulphur bacteria. Non-filamentous.

1. *Rhodomonas.* (*Chromatium*)
2. *Rhabdomonas.* (*Rhabdochromatium*)
3. *Rhododictyon.* (*Thiodictyon*)
4. *Amoebomonas.* (*Amoebobacter*)
5. *Rhodothece.* (*Thiothece*)
6. *Rhodopolycoccus.* (*Thiopolycoccus*)
7. *Rhodococcus.* (*Thiopedia*)
8. *Lamprocystis*
9. *Rhodocystis.* (*Thiopedia*)
10. *Rhodocapsa.* (*Thiocapsa*)
11. *Rhodosarcina.* (*Thiosarcina*)
12. *Rhodospirillum.* (*Thiospirillum*)

(Genera as defined by Winogradsky and Molisch)

V. Family Trichobacteriaceae

Filamentous water bacteria. All iron bacteria except *Cladothrix* and *Spirochaeta.*

1. *Cladothrix.* False branching, no sulphur granules
2. *Crenothrix.* No branching or sulphur granules Attached straight filaments)
3. *Beggiatoa.* Sulphur present. Straight unattached motile filaments
4. *Thiothrix.* Same as preceding but attached
5. *Leptothrix.* Unbranched straight unattached filaments. No sulphur
6. *Spirophyllum.* Flattened spiral filaments. No branching. No sulphur
7. *Spirochaeta.* Slender and flexuous spiral filaments. No branching. No sulphur

VI. Family Luminibacteriaceae

Phosphorescent and fluorescent forms. Denitrifiers.

1. *Denitromonas.* Non-liquefying, actively-dentrifying rods, usually fluorescent

2. *Liquidomonas.* Liquefiers
3. *Liquidovibrio.* Spiral forms
4. *Liquidococcus.* Liquefying cocci
5. *Solidococcus.* Non-liquefying cocci

VII. Family Reducibacteriaceae

Spiral forms, actively reducing sulphates.

1. *Solidovibrio.* Short spirals. Non-liquefying
2. *Spirillum.* Longer spirals

B. Order, *Peritrichinae*

Peritrichic when flagella are present. Rods or cocci. Not typically water bacteria. Splitting of carbohydrates or amino acids plays primary rôle in metabolism rather than oxidation or denitrification.

I. Family Acidobacteriaceae

Aerobic or facultative anaerobic. Metabolism of carbohydrates predominant, with production of acids.

1. *Denitrobacterium.* Actively-denitrifying forms
2. *Bacterium.* Rod forms which actively ferment carbohydrates and form gas. Non-liquefying
3. *Propionibacterium.* Forms which produce propionic acid. Non-motile. Do not attack casein. Form hydrogen sulphide from peptone
4. *Caseobacterium.* Attack casein. Do not form gas. Non-motile. Grow best under anaerobic conditions
5. *Streptococcus.* Chains of cocci. Usually non-liquefying. Slime formation common
6. *Micrococcus.* Irregular groups of cocci. Usually liquefying
7. Packet forms

II. Family Alkalibacteriaceae

Aerobic or facultative anaerobic. Metabolism of nitrogenous compounds predominant, with production of ammonia.

1. *Liquidobacterium.* Non-spore-forming. Proteus-like bacteria
2. *Bacillus.* Usually form spores. Typically ferment carbohydrates, and reduce nitrogenous compounds with production of much ammonia. Often form diastase. Usually not indol
3. *Urobacillus.* Decompose urea and require special alkaline media for development. Usually non-liquefying

III. Family Butyribacteriaceae

Typically anaerobic spore formers. Acting largely upon carbohydrates, forming acids, particularly butyric acid.

1. *Butyribacillus.* Acting primarily upon sugars, forming butyric acid. Usually clostridia
2. *Pectobacillus.* Acting primarily upon pectins. Usually liquefiers
3. *Cellulobacillus.* Acting primarily upon cellulose. Plectridia

IV. Family Putribacteriaceae

Typically anaerobic spore formers. Acting largely upon proteins, bringing about putrefactive changes.

1. *Putribacillus.* Not forming an exotoxin
2. *Botulobacillus.* Producing an exotoxin. Plectridia

II. PRINCIPLES OF BOTANICAL NOMENCLATURE

The chief aim of terminology is to give an object,—in biology a kind of animal or plant—a single definite name. So important is this that both botanists and zoologists have, in international congress, established rules of nomenclature designed to attain it. Without such rules agreement as to nomenclature is impossible, and although the

rules so formulated are often arbitrary and sometimes complex, they are the result of careful study, and it is not likely that we as bacteriologists can hope to improve upon the work of trained and experienced systematists in other biological lines.

Many workers in medical bacteriology and in other special fields of applied micro-biology, who deal with only a few well-recognized species, may perhaps feel no need for any change in current practice. Few can deny, however, that it is a serious inconvenience for such names as *B. Welchii, B. sporogenes, B. perfringens,* to be used by various workers, sometimes for the same, sometimes for different organisms, or for the same form to be described as *Bacterium lactis aerogenes* or *Streptococcus lacticus* when it is isolated from milk and as *Streptococcus salivarius* or *Str. fecalis* when it is isolated from the human mouth or intestine.

When one passes from a study of the practical effects of the activity of some particular microbe to a consideration of its relationship to other forms it becomes essential, not only to have a name for each kind of organism, but to have also a system of nomenclature which will make it possible to express such relationships with reasonable clearness and accuracy.

This need is met by the Linnaean system of classification universally adopted by all biologists outside of our own limited and systematically undeveloped field. According to this system each recognizable kind of plant or animal receives a binomial Latin name, the first half designating the genus or group to which it belongs, and the second half the particular kind or species to which the name applies. The genera in turn are grouped in tribes, the tribes in families, the families in orders and the orders in classes. These divisions will often be artificial and often of course of very unequal size and importance in different groups. They make it possible, however, to express in a simple manner the essential facts of biological relationship,—the fact that A and B and C are more nearly related to each other than are any of them to D and E and F; and that the series A-F exhibits common relationships closer than any similarities which its members bear to G or H.

If such a system is accepted it is in the next place important to make sure that each group, from species to class, shall bear a single universal name. The name need not be appropriate; it need only be stable. It is an arbitrary label, not a description. If the door be once opened to criticism on the ground of inappropriateness stability of course must disappear.

It is in order to ensure uniformity and stability of nomenclature

that the International Codes referred to have been formulated; and it is to the International Rules of Botanical Nomenclature[1] (1910) that we as bacteriologists should naturally turn for guidance.

Leaving out a great many minor rules and recommendations, the most important of the rules which would affect bacteriological practice may be cited as follows.

Chap. I, Art. 7. "Scientific names are in Latin for all groups."

Chap. II, Art. 10. "Every individual plant belongs to a species (species), every species to a genus (genus), every genus to a family (familia), every family to an order (ordo), every order to a class (classis), every class to a division (divisio)."

Chap. III, Sect. 1, Art. 15. "Each natural group of plants can bear in science only one valid designation, namely the oldest, provided that it is in conformity with the rules of Nomenclature and the conditions laid down in articles 19 and 20 of Section 2."

[1] "The International Rules of Botanical Nomenclature, Adopted by the International Congresses of Vienna, 1905 and Brussels, 1910" were published in French, German, and English in the same volume by Gustav Fischer, Jena, 1912. The discussion of the 1905 Rules in Rhodora (Vol 9, no. 99, pp. 29–52, March, 1907, Preston & Rounds Co., Providence, R. I.) is more easily obtained, and serves the main needs of those interested in taxonomy.

The American Code of Botanical Nomenclature, published in the Bulletin of the Torrey Botanical Club, (Vol. 34, pp. 167–178, 1907) is used by all but one of the important botanical centers in the United States. It is a briefer, simpler set of rules formulated by an American Commission, because of certain objections to the Vienna rules. This Commission characterizes as "the height of arbitrary action" the Vienna ruling that descriptions of all new species or genera must be accompanied by a diagnosis in the Latin language. That demand was voted down also by our Society at the New Haven meeting in 1916. The contention that the "Vienna Congress failed to recognize the principle of types" affects bacterial taxonomy less than other divisions of taxonomy; type specimens cannot be preserved as with larger plants; when possible, however, the type species of a genus has been given in the following classification. The third objection of the American Commission was to the large "conservenda" list of the Vienna Congress.

The small size of bacteria, the lack of satisfactory distinguishing morphological characters, and the incompleteness and indefiniteness of the early taxonomic characterizations make it impossible to select for bacteria a workable "point of departure" (such as Species Plantarum, 1753, for flowering plants) without losing all the old classic names in the sense in which they are generally understood. Having, therefore, in this classification attempted to *conserve* these old names, it has seemed wiser to quote from the Vienna Code, especially as it is better known among bacteriologists abroad.

Chap. III, Sect. 2, Art. 19. "Botanical nomenclature begins for the different groups of plants (recent and fossil.)"

Chap. III, Sect. 2, Art. 20. "However, to avoid disadvantageous changes in the nomenclature of genera by the strict application of the rules of nomenclature, and especially of the principle of priority in starting from the dates given in Art. 19, the rules provide a list of names which must be retained in all cases. These names are by preference those which have come into general use in the fifty years following their publication, or which have been used in monographs and important floristic works up to 1890."

Chap. III, Sect. 3, Rec. III. "Orders are designated preferably by the name of one of the principal families, with the ending *ales*."

Chap. III, Sect. 3, Art. 21. "Families (familiae) are designated by the name of one of their genera or ancient generic names with the ending *aceae*."

Chap. III, Sect. 3, Art. 23. "Names of subfamilies (subfamiliae) are taken from the name of one of the genera in the group, with the ending *oideae*. The same holds for the tribes (tribus) with the ending *eae* and for the subtribes (subtribus) with the ending *inae*."

Chap. III, Sect. 3, Art. 24. "Genera receive names, substantives (or adjectives used as substantives) in the singular number and written with a capital letter which may be compared with our own family names. These names may be taken from any source whatever and may even be composed in an absolutely arbitrary manner."

Chap. III, Sect. 3, Art. 25, Rec. V. "Botanists who are publising generic names show judgment and taste by attending to the following recommendations:

"a. Not to make names very long or difficult to pronounce.

"b. Not to use again a name which has already been used and has lapsed into synonymy (homonym).

"c. Not to dedicate genera to persons who are in all respects strangers to botany or at least to natural science, nor to persons quite unknown.

[2] The date for the beginning of recognized nomenclature for the *Schizomycetes*(*Bacteria*) was left to be settled by the International Botanical Congress which was to have been held at London in 1915, but was postponed on account of the War. For the algae, 1753 was selected, but *six* additional later dates for various algal families &c. are added. Adoption of this classification in its final form would seem less arbitrary and much less confusing than having seven points of departure, as in the algae.

"d. Not to take names from barbarous tongues, unless those names are frequently quoted in books of travel, and have an agreeable form that is readily adapted to the Latin tongue and to the tongues of civilized countries.

"e. To recall, if possible, by the formation or ending of the name, the affinities or the analogies of the genus.

"f. To avoid adjectives used as nouns.

"g. Not to give a genus a name whose form is rather that of a subgenus or section (e. g. Eusideroxylon, a name given to a genus of Lauraceae, which, however, being valid, cannot be changed).

"h. Not to make names by the combination of two languages (nomina hybrida)."

Chap. III, Sect. 3, Art. 26. "All species, even those that singly constitute a genus, are designated by the name of the genus to which they belong, followed by a name (or epithet) termed specific, usually of the nature of an adjective (forming a combination of two names, a binomial, or binary name)."

Chap. III, Sect. 3, Art. 26, Rec. VIII. "The specific name should, in general, give some indication of the appearance, the characters, the origin, the history or the properties of the species. If taken from the name of a person, it usually recalls the name of the one who discovered or described it, or was in some way concerned with it."

Chap. III, Sect. 3, Rec. X. "Specific names begin with a small letter except those which are taken from names of persons (substantives or adjectives) or those which are taken from generic names (substantives or adjectives)."

Chap. III, Sect. 3, Rec. XIV. "In forming specific names, botanists will do well to note the following recommendations:

"a. Avoid very long names and those which are difficult to pronounce.

"b. Avoid names which express a character common to all or nearly all of the species of the genus.

"c. Avoid names taken from little known or very restricted localities, unless the species be very local.

"d. Avoid, in the same genus, names which are very much alike, especially those which differ only in their last letters.

"e. Adopt unpublished names found in traveller's notes and herbaria, attributing them to the authors concerned, only when those concerned have approved the publication.

"f. Avoid names which have been used before in the genus, or in any closely allied genus, and which have lapsed into synonymy (homonyms).

"g. Do not name a species after a person who has neither discovered, nor described, nor figured, nor in any way studied it."

"h. Avoid specific names formed of two words.

"i. Avoid names which have the same meaning as the generic name."

Chap. III, Sect. 4, No. 35. "Publication is effected by the sale or public distribution of printed matter or indelible autographs. Communication of new names at a public meeting, or the placing of names in collections or gardens open to the public, do not constitute publications."

Chap. III, Sect. 4, Art. 36. "On and after January 1, 1908, the publication of names of new groups will be valid only when they are accompanied by a Latin diagnosis."[3]

Chap. III, Sect. 4, Art. 37. "A species or a subdivision of a species, announced in a work, with a complete specific or varietal name, but without diagnosis or reference to a former description under another name, is not valid. Citation in synonymy or incidental mention of a name is not effective publication." . . . .

Chap. III, Sect. 4, Art. 38. "A genus or any other group of higher rank than a species named or announced without being characterized conformably to Article 37 cannot be regarded as effectively published (nomen nudum)." . . . .

Chap. III, Sect. 5, Art. 40. "For the indication of the name or names of a group to be accurate and complete, and in order that the date may be readily verified, it is necessary to quote the author who first published the name or combination of names in question."

Chap. III, Sect. 5, Art. 41. "An alteration of the constituent characters or of the circumscription of a group does not warrant the quotation of another author than the one who first published the name or combination of names."

Chap. III, Sect. 6, Art. 44. "A change of characters, or a revision which involves the exclusion of certain elements of a group or the addition of new elements, does not warrant a change in the name or names of a group except in cases provided for in Article 51.

Art. 45. "When a genus is divided into two or more genera, the name must be kept and given to one of the *principal* divisions,—

[3] Not accepted by this Society at the New Haven meeting, December, 1916.

either to the division containing the *type* of the original group or to the division containing the largest number of a species."

Art. 46. "When two or more groups of the same nature are united, the name of the oldest is retained."

Art. 47. "When a species or subdivision of a species is divided into two or more groups of the same nature, if one of the two forms was distinguished or described earlier than the other, the name is retained for that form."

Art. 48. "When a subgenus or section or species is moved into another genus, when a variety or other division of a species is moved into another species, retaining there the same rank, the original name of the subgenus or section, the first specific epithet, or the original name of the division of the species must be retained or must be re-established, unless, in the new position there exists one of the obstacles indicated in the articles of section 7.

Art. 49. "When a tribe becomes a family, a subgenus or a section becomes a genus, a subdivision of a species becomes a species, or the reverse of these changes takes place, and speaking generally when a group changes its rank, the earliest name (or combination of names) received by the group in its new position must be regarded as valid, if it is in conformity with the rules, unless there exist any of the obstacles indicated in the articles of section 7."

Art. 50. "No one is authorized to reject, change or modify a name (or combination of names) because it is badly chosen, or disagreeable, or another is preferable or better known, or because of the existence of an earlier homonym which is universally regarded as non-valid, or for any other motive either contestable or of little import." (See also art. 57.)

Art. 51. "Every one should refuse to admit a name in the following cases:

"1. When the name is applied in the plant kingdom to a group which has an earlier valid name.

"2. When it duplicates the name of a class, order, family or genus, or a subdivision or species of the same genus, or a subdivision of the same species.

"3. When it is based on a monstrosity.

"4. When the group which it designates embraces elements altogether incoherent, or when it becomes a permanent source of confusion or error.

"5. When it is contrary to the rules of sections 4 and 6."

Art. 53. "When a species is moved from one genus into another, its specific epithet must be changed if it is already borne by a valid species of the genus." The same rule applies to other transferred groups.

Art. 56. "In the cases foreseen in articles 51 to 56, the name to be rejected or changed is replaced by the oldest valid name in the group in question, and in default of such a one a new name must be made."

Chap. IV, Art. 58. "The rules of botanical nomenclature can only be modified by competent persons at an international congress convened for the express purpose."

In general these rules and the others not here cited seem to us entirely reasonable and well suited to bring some order into the chaos of bacteriological nomenclature, as they have done in other botanical fields. The requirement of a Latin diagnosis seems, however, an *unnecessary* one, and it is certainly a requirement which would not be readily accepted by working bacteriologists.

The question as to the date at which valid bacteriological nomenclature shall be considered to begin is one of fundamental importance in the application of this system. As noted above, the International Botanical Congresses have established such dates for the various groups of higher plants, but not as yet for the bacteria. Erwin F. Smith (1905), in his suggestive discussion of the general problems of bacterial classification, urges that all names suggested prior either to 1872 (the date of publication of Cohn's classic paper) or to 1881 (the date of the introduction of solid culture media) should be considered *nomina nuda*. With the latter suggestion we are in general in accord. The characterizations of bacteria before this date were almost of necessity based upon mixed cultures, and the descriptions are vague and puzzling to a degree. Furthermore, the acceptance of these earlier descriptions would rob us of almost all our familiar names, and give us strange generic and specific names which it would be impossible ever to force upon workers in applied bacteriology. The introduction of a simple method of making pure cultures marks a natural point of departure for bacterial classification; but a slightly later date, 1885, seems to form a better point of departure, since the important contribution which appeared at that time, Zopf's Spaltpilze, established a group of bacterial genera which are for the most part natural ones, and which bear names familiar to all bacteriologists down to the present day.

We therefore recommend to the Society of American Bacteriologists:

(a) That the International Rules for Botanical Nomenclature be accepted by the Society as governing bacterial terminology, with the exception that French, English or German may be substituted for Latin in the diagnosis.

(b) That the date of publication of the third edition of Zopf's Spaltpilze be considered as the date for the beginning of bacteriological nomenclature for the purpose of determining priority, with the exception of a list of genera conservanda to be adopted by the Society at its 1918 meeting.

(c) That the Society take steps to present these recommendations to the next International Botanical Congress, and if possible to secure favorable action thereon by that body.

III. SYSTEM OF CLASSIFICATION RECOMMENDED BY THE COMMITTEE: FAMILIES

It has seemed evident to the Committee that none of the systems of classification outlined above gives a satisfactory picture of the real relationships of bacterial types. The classifications by Zopf and by Lehmann and Neumann are good as far as they go; these earlier classifications might be helpfully supplemented by Kruse's groups of bacilli and by the Winslows' genera of cocci. Many of the distinctions in Jensen's outline are suggestive and important. Each of the general systems proposed has, however, been limited in important respects. The earlier workers ignored physiological differences, while Jensen gave to certain of them a quite arbitrary and artificial importance. We are of the opinion that the facts at hand warrant a review of the whole question, and the working out of a new system of classification which shall include what is valid and discard what is arbitrary in the older classifications—with no idealistic conceptions, either morphological or physiological in mind—but with the sole purpose of recognizing and defining the principal groups of bacteria which exhibit circumstantial evidence of common evolutionary relationship.

The conditions which call for reform, and the principles which should govern them, have been admirably stated by Jordan (1914) as follows:

The present nomenclature of bacteriology may be criticized on two grounds: first, as already pointed out, for the unwieldy size that certain "genera" have been allowed to assume; and, second, for the haphazard way in which trinomial and even quadrinomial names have been bestowed. Such names can be properly employed only with reference to subspecies or varieties; and designations like *B. coli communis*, *Granulobacillus saccharobutyricus mobilis non-liquefaciens*, and *Micrococcus acidi paralactici liquefaciens Halensi*, are both cumbersome and unscientific. The use of a single genus name for a multitude of organisms is, in fact, responsible for the tendency toward trinomial nomenclature, and the remedy for both conditions would seem to lie in the abandonment of such a term as *Bacillus* for the name of a genus and the frank establishment of new genera on the basis of physiologic characters: such, for example, as distinguish the colon-typhoid group or the diphtheria group of bacilli. Until some such reform in nomenclature is brought about the names used to designate different kinds of bacteria will fail to make clear the group relationships which undoubtedly exist, and will continue to be a stumbling block to all students of the subject.

The first point which must be considered in formulating a system of bacterial classification is the limitation of the general field to be considered—the definition of the class Schizomycetes. There are three groups in particular whose relationships are doubtful, the Myxobacteriaceae, the *Sporothrix* forms, and the Spirochaetes.

The Myxobacteriaceae are included by Smith (1905) as a family of the Schizomycetes, but we cannot feel that such a step is justified. The peculiar life cycle of these forms with their amoeboid stage and rather complex cyst production seems to separate them quite widely from the other bacteria. They should certainly constitute a distinct order, the Myxobacteriales but they may conveniently be included within the class Schizomycetes.

The *Sporothrix* forms are much more sharply differentiated from the bacteria by their much-branched septate mycelium and their characteristic spores. They undoubtedly belong with the higher fungi.

The position of the Spirochaetes is much more uncertain. The character of the disease processes excited by these organisms as well as their relation to certain chemicals and the presence in certain forms of the so-called "undulating membrane," suggest a protozoan nature. The character of the flagella, the rapid multiplication by transverse division and the behavior of these organisms in plasmolysis are on the other hand distinctively bacterial characters. It is certainly a straining of the facts to place these forms in the family Spirillaceae, as if they were closely related to *Vibrio* and *Spirillum*. The best course seems to be to consider them as forming a distinct family intermediate between the bacteria and the Protozoa.

The next problem to be considered concerns the relation between the higher filamentous bacteria and the ordinary types. We are inclined in this matter to follow Zopf and the earlier workers in separating these organisms rather widely, rather than Kruse and Jensen who group them together. The red and the colorless sulphur bacteria and the iron bacteria differ fundamentally in metabolism from the ordinary bacteria; and these types as well as the *Cladothrix* forms have a quite characteristic morphology and habitat.

Almost all systematists have separated the purple bacteria from the ordinary types, and both Zopf and Migula gave the other filamentous forms at least distinct family rank. The classification most commonly adopted is that of Migula, which separates the sulphur bacteria as a distinct order and includes the iron bacteria, the *Cladothrix* types, and even the branched Sphaerotilus forms with the ordinary bacteria, but in a separate family, Chlamydobacteriaceae. It seems to us that the broad difference between all these filamentous oxidative water bacteria and the types we cultivate in the laboratory is far more fundamental than that between, for example, the spherical and the rod-shaped bacteria. We suggest that the filamentous types, which are not sulphur users, should be placed in the Chlamydobacteriales, defined as below, while the sulphur forms should be placed in a separate order, Thiobacteriales. In this we follow Buchanan (1917).

We have not attempted to discuss the genera of these two series, as they are beyond the scope of the work of the ordinary bacteriologist. The orders Chlamydobacteriales and Thiobacteriales as defined belong largely to the botanist, the group of the Eubacteriales with its cultivable forms, studied by a special physiological technique, to the bacteriologist.

Jensen's division into the orders Cephalotrichinae and Peritrichinae places the filamentous bacteria with the oxidizing bacteria, the Actinomycetes and the phosphorescent and reducing forms, and separates all these groups from the bacteria which possess peritrichic flagella and utilize carbohydrates and amino-acids. There are undoubtedly real differences between Jensen's two main groups, but we are inclined to the opinion that the first, second, sixth and seventh families of his Cephalotrichinae are much more closely related to the Peritrichinae than to the filamentous water bacteria, and that the differences in metabolism and arrangement of flagella on which he bases his division are of family rather than ordinal rank.

Taking the order Eubacteriales as including all the Schizomycetes except the *Cladothrix*, *Crenothrix*, *Beggiatoa* and purple bacteria types, the next problem is a division of this order into natural family groups.

Of the three classic bacterial families, the Coccaceae, the Spirillaceae, and the Bacteriaceae, the first two seem to us to represent fairly distinct entities which should be preserved intact. They appear in the classifications of Hueppe, Lehmann and Neumann, Migula, and Kruse, and the breaking up of the distinction between these types and the rod-shaped bacteria by Jensen seems to us a distinct step backward. It is not of course the single morphological character of roundness or twistedness that is so important, but the fact that both the cocci and the spiral bacteria (except the Spirochaetes) show a number of common characters which appear to us to indicate real relationship.

The Actinomycetes group, established by Lehmann and Neumann for the diphtheria, tuberculosis and actinomyces types, seems to us a good family,—characterized by the branching of the cells, frequent occurrence of clubbed forms, the ab-

sence of motility and the rather slow, often mold-like growth on media. This family was properly named Mycobacteriaceae by Chester. The fusiform bacilli may be placed here, for the present, at least.

The classic family Bacteriaceae seems to us to be the group which needs most radical revision. It includes a great complex of organisms differing from each other radically in structure and metabolism, and might conveniently be split up into at least five distinct families.

First of all it seems to us that the group of the spore formers, distinguished as the genus *Bacillus* by Zopf, is a sufficiently clearly marked group to deserve family rank. The power of spore formation in itself is a rather fundamental one, and its combination with a very complex metabolism, Gram-positive staining, and the peritrichic arrangement of flagella, when present, marks off the Bacillaceae as one of the highest groups of the bacteria.

The next group of rod-shaped organisms which deserves special recognition is the group characterized by a very primitive metabolism,—life energy being derived mainly from simple oxidative processes without the splitting of carbohydrates or the metabolism of amino-acids. This is Jensen's Oxydobacteriaceae, including the types which oxidize methane, carbon monoxid and hydrogen, the acetic acid bacteria, the nitrosifiers and nitrifiers, and the *Azotobacter* type. Unfortunately the name given by Jensen cannot stand in face of the rule of botanical nomenclature that a family name must be formed from one of its component genera with the suffix *aceae*. We suggest the name Nitrobacteriaceae for this family.

A third group of rod-shaped bacteria which seems to us worthy of family rank is the group which roughly corresponds to the rod forms in Jensen's family, Luminibacteriaceae, with the addition of other types, not fluorescent or phosphorescent, which seem closely allied to the former. This group should provisionally include all the forms (except certain genera of Nitrobacteriaceae) which possess polar flagella, this primary characteristic being associated as a rule with a limited carbohydrate metabolism

and active oxidative action. The common types of this family are either water bacteria or plant pathogens. The family should be called *Pseudomonadaceae* from Migula's genus.

A fourth family should perhaps be recognized for the high acid formers of the *B. acidophilus* and *B. bifidus* and *B. bulgaricus* types. The peculiar morphology and the unique acid tolerence of these organisms seem to warrant family rank, and we suggest the generic name *Lactobacillus* and the family name Lactobacillaceae.

The rest of the rod-shaped bacteria may for the present most wisely be left in the family Bacteriaceae. The typical forms in this family are those of the compact colon-typhoid group, with their peritrichic flagella and complex metabolic powers. The *B. cloacae* types, the *Proteus* forms and the peritrichic water bacteria grade through *B. aerogenes* into the colon-typhoid series. Jensen's groups of the *Acido-* and *Alkalibacteriaceae* do not seem to us of family rank.

The family Bacteriaceae must necessarily remain somewhat of an *omnium gatherum* from which other families will no doubt be split off as knowledge advances. We have hesitated however to burden the literature with unnecessary terminology, and prefer to leave these forms in the family Bacteriaceae until further study indicates that a sharper differentiation is desirable.

IV. SYSTEM OF CLASSIFICATION RECOMMENDED BY THE COMMITTEE: GENERA

We may now proceed to a brief review of the eight families proposed, and of their generic subdivisions.

The Nitrobacteriaceae are clearly the most primitive of the Eubacteriales. Their power to live without complex organic substances would have made it possible, as Jensen points out, for them to flourish at a very early period in the world's history, and their simple structure is in harmony with the view that they represent the ancestral type of all other bacteria.

The three genera suggested by Jensen for the types which derive their life energy from the oxidation of methane, carbon monoxid and hydrogen respectively, include only a few rare and

little-studied forms. Their metabolism is, however, so unique as perhaps to warrant the recognition of the genera, *Methanomonas*, *Carboxydomonas* and *Hydrogenomonas*. His four other genera, which respectively include the acetic acid bacteria, the nitrosifiers, the nitrifiers and the nitrogen fixers of the soil, are clearly distinct genera; but they should bear the old names, *Mycoderma*, *Nitrosomonas*, *Nitrobacter*, and *Azotobacter*, not the new names unwarrantably coined by Jensen.

The second family to be considered is that of the Mycobacteriaceae, including a series of organisms which show a definite tendency to branch, leading finally to the complex mycelia of *Actinomyces*, which exhibit marked peculiarities in staining reactions, and which show in the three most typical genera a peculiar kind of localized pathogenicity leading to the production of local swellings of host tissue.

All of these organisms are strongly aerobic and oxidative forms, and *Actinomyces* can derive its nitrogen from nitrates, although not from free nitrogen. These are primitive characteristics, but the Gram-positive staining and the difficult growth of the pathogenic types on media indicate a considerable degree of differentiation. Theobald Smith has pointed out that the tubercle bacillus represents a peculiarly high type of parasitic adaptation, which is natural if it is derived, as we believe, from one of the earliest bacterial groups.

We may perhaps consider *Rhizobium* as connecting the Nitrobacteriaceae and Mycobacteriaceae, *Rhizobium* deriving its origin from *Azotobacter*. In Mycobacteriaceae we may place Lehmann and Neumann's genus, *Mycobacterium*, including the tubercle and pseudotubercle forms, the leprosy organism, and possibly the glanders bacillus. This organism is commonly classed with the tubercle bacillus, which it resembles in its branching cells and slow growth on media and in certain phases of its pathogenesis. Its Gram-negative staining reaction is a peculiar feature in this otherwise Gram-positive group. This genus clearly is very close to *Actinomyces*, which in turn forms a connecting link with the higher molds, as indicated by the tendency to branching and the formation of conidia.

The genus, *Corynebacterium*, including the diphtheria and diphtheroid groups, is related to this family by the tendency to cell branching and irregular staining, by its positive Gram reaction, and its oxidative tendencies. On another side it is allied in many respects with the streptococci, and very possibly may have formed a connecting link with this group.

With some doubts, we have placed the fusiform bacilli in this group as the genus *Fusiformis* (Holling). They resemble the members of the family Mycobacteriaceae in their tendency to filament formation and irregular staining, in their Gram-positive reaction and lack of motility. They of course differ markedly in their anaerobic and essentially putrefactive metabolism; but seem on the whole to fit better here than anywhere else.

Another distinctly primitive family is that of the Pseudomonadaceae. The polar flagella, the oxidative metabolism and active production of ammonia with very limited utilization of carbohydrates, and the predominant water habitat are all primitive characteristics. Both the fluorescent water bacilli and many plant pathogens like *Ps. campestris* may for the present be grouped in a single genus. If no limit were recognized for the beginning of adequate bacteriological nomenclature the name of this genus, as pointed out by Smith (1905), must be *Bacterium*, for both Ehrenberg (1838) and Cohn (1872) used this term for a form with terminal flagella. If, however, names proposed prior to 1881 are considered as *nomina nuda* it is proper to use the term Bacterium as defined by Zopf for the main group of non-spore-forming bacteria, and Migula's later differentiation of the types with polar flagella becomes valid. This genus, it may be noted, includes one form which in a limited degree is pathogenic for man, *Ps. pyocyanea*.

The next family to be considered is that of the spiral bacteria (excluding the Spirochaetes). The vibrios and spirilla, by their polar flagella, simple metabolism, (primarily oxidative, with formation of ammonia, rather than fermentative) and water habitat, are clearly allied to the *Pseudomonas* types.

They form two fairly distinct morphological genera, *Vibrio*, which includes the comma forms with a single terminal flagellum, and *Spirillum*, which includes the large lophotrichic water spirilla. Migula (1896) calls the first of these genera *Microspira*, Schroeter. Smith (1905) has pointed out that if no time limit be placed on bacterial terminology, Trevisan's name *Pacinia* antedates *Microspira* and must supplant it. If, on the other hand, we exclude names suggested prior to 1881 Zopf's use of the name *Vibrio* will stand.

We see no good reason for recognizing a non-motile genus, *Spirosoma*, as Migula does, the loss of motility being presumably a character of very slight importance here as in other bacterial groups. It is evident that the spirochaetes are quite widely separated by structure and parasitic habit from the other genera of the spiral bacteria. It may well be that these forms do not belong among the bacteria at all. We have placed them for the present in a separate family at the end of our system.

We come next to the spherical bacteria, which make up a fairly well-defined family including two main series, one saprophytic, the other parasitic. The first is allied, through the water micrococci, to *Pseudomonas*, and the second, through the streptococci, to *Corynebacterium* and possibly to the colon-typhoid group.

In the subdivisions of this family we have, for the most part, followed the revision of the Winslows (1908) with the changes in terminology suggested by Buchanan (1915). We are inclined to think, however, that in the light of more recent investigations the genus *Diplococcus* as defined by the Winslows is an artificial one. It seems to us that the pneumococcus is more closely allied to the streptococci than it is to the Gram-negative, biscuit-shaped gonococci and meningococci. We are also doubtful of the validity of the genus, *Ascococcus* (*Leuconostoc*). For the present we have omitted it from our scheme, and we have re-defined *Streptococcus* so as to include the pneumococci and the zooglea-forming types, as well as the true chain formers.

Following the Winslows, the white staphylococci, characterized by more abundant surface growth and less vigorous gelatin liquefaction, as well as by the lack of orange pigment, are separated from *Staphylococcus* in a separate genus, *Albococcus*. Among the Gram-negative, feebly fermenting saprophytic forms, the yellow types are placed in *Micrococcus* and *Sarcina*, the red types in *Rhodococcus*. The mere presence or absence of packets, as the Winslows point out, is a slender basis for a distinction between the first two of these genera, but it is perhaps best to retain a name which has nearly a century of usage behind it until more definite proof of its invalidity is forthcoming.

The seventh family, the Bacteriaceae, must for the present include all the non-spore-forming, Gram-negative rods which when motile possess peritrichic flagella. Besides several lesser genera, there are two principal series here, one parasitic and often pathogenic, the other saprophytic. The first group, which may conveniently bear the old generic name *Bacterium*, is the colon-typhoid-dysentery group.

If the names of organisms imperfectly described in the early days of bacteriology were to be accepted as valid the name *Bacterium*, as pointed out above, would have to be reserved for the forms with polar flagella, leaving the great mass of non-spore-forming bacteria to be designated by some new and unfamiliar name, or by some very old and equally unfamiliar one which might be dug out of the literature. The fact that this group includes many of the common pathogenic forms universally designated by medical workers under one of the names *Bacillus* or *Bacterium*, would make it peculiarly unfortunate to introduce new terminology. If, however, we consider names proposed prior to 1885 as *nomina nuda*, the use by Zopf of the term *Bacterium* for the non-spore-formers becomes valid and it is permissible to retain this name for the group under consideration. *B. abortus* may for the present be left in this genus in spite of its peculiar oxygen relations. Here also the proteus and aerogenes series and the chromogenic water bacteria may be left for the present.

On the other hand the plant pathogens, such as *B. amylovorus*, *B. phytophthorus*, *B. caratovorus*, *B. melonis*, and *B. solanacearum* seem to us perhaps to deserve distinct generic rank. They resemble the proteus group in their Gram-negative reaction, general growth characters, tendency to slime formation, and generally negative indol reaction, but their fermentative powers are less vigorous and the exact biochemical changes they produce in carbohydrates are unknown. We suggest that all the peritrichiate plant pathogens be for the present grouped together under a new genus to be called *Erwinia*, after our American Pasteur of bacterial plant pathology, Erwin Smith.

Two other groups of the Bacteriaceae seem to us to deserve distinct generic rank. The hemorrhagic septicemia organisms by their bipolar staining and characteristic pathogenesis may properly be grouped under the name *Pasteurella*, including in the genus the causative organisms of fowl cholera, swine plague, rabbit septicemia, hemorrhagic septicemia of cattle and bubonic plague.

The other group, for which we suggest the generic name, *Hemophilus*, should include the small non-motile parasites, which will grow only on media in the presence of blood or other body fluids. The influenza bacillus, the Koch-Weeks bacillus and the Bordet-Gengou bacillus are the principal representatives of this group.

The peculiarities of the high acid-forming Gram-positive bacteria of the *B. bulgaricus*, *B. acidophilus* and *B. bifidus* types seem so significant as to warant the creation for them of a distinct family, the *Lactobacillaceae*.

Finally, the last and probably the most complex family is that of the Bacillaceae, which includes the large Gram-positive rods which form endospores and actively decompose protein media through the agency of enzymes. These organisms are clearly related to each other, but include two distinct series, one aerobic, the other anaerobic. The aerobic types of the *B. subtilis* and *B. megatherium* series, with *B. anthracis* and B. *lactimorbi*, may properly bear the ancient name *Bacillus* applied to them by Zopf, while the anaerobic, rod-shaped types

should properly bear the name *Clostridium* applied by the same author to those of them which showed a spindle-shaped enlargement at spore formation. It should be understood, however, that we define the genus by its anaerobic properties, and include in it plectridium and clostridium forms and those which show no enlargement at spore formation. It is quite possible that this group may later be subdivided, but we prefer to err on this side rather than unduly to complicate terminology. The bacillus of malignant oedema may for the present be left in this genus, although it is said to show a Gram-negative reaction.

In regard to the genera to be recognized in the family Spirochaetaceae—which, as pointed out above, must be considered an aberrant group intermediate between bacteria and protozoa—we have consulted with Prof. G. N. Calkins of Columbia, and in the outline below have accepted the characterization of the four genera, *Spirochaeta*, *Cristispira*, *Saprospira*, and *Treponema* as given by him.

The families and genera listed below seem to us to represent the minimum number of bacterial groups which must be recognized in order to embody our present knowledge of bacterial relationships; and we submit it in the hope that it may serve as a basis for the future development of classifications which shall more and more closely approximate the facts of evolutionary history.

It is our hope that the present report of the Committee may be put in type and distributed to the members of the Society in proof form for careful consideration. After all suggestions in connection with it have been fully considered a revised report may be presented next year for formal adoption.

If this plan can be carried out we earnestly bespeak the support of the individual members for such a modification of the classification here presented as the Society may finally adopt. It will of course be subject to constant change with advancing knowledge; but such changes should be based on careful systematic study and carried out in accordance with recognized rules of nomenclature. A single clearly defined system of families and genera must form the starting point for progress.

Such a system the Society should be able to agree upon after a year's consideration. A formal endorsement will be of little value, however, unless the names suggested are used in actual practice, in written papers and in the teaching of the class-room. It is easy to continue in the old slipshod ways, and there will be a powerful force of inertia to overcome. If, however, this Society can actually bring into use a system of classification which approximately represents natural bacterial relationships and terminology which is uniform and in accord with the rules of priority, a great service will have been rendered to the science of bacteriology.

V. SUGGESTED OUTLINE OF BACTERIAL CLASSIFICATION

THE CLASS SCHIZOMYCETES

Minute, one-celled, chlorophyll-free, colorless, rarely violet-red or green-colored plants, which typically multiply by dividing in one, two or three directions of space, the cells thus formed sometimes remaining united into filamentous, flat, or cubical aggregates. Filamentous species often surrounded by a common sheath. Capsule or sheath composed in the main of protein matter. The cell plasma generally homogeneous without a nucleus. Sexual reproduction absent. In many species resting bodies are produced, either endospores or gonidia. Cells may be motile by means of flagella.

A. ORDER MYXOBACTERIALES[4]

Cells united during the vegetative stage into a pseudoplasmodium which passes over into a highly-developed cyst-producing resting stage.

B. ORDER THIOBACTERIALES[4]

Cells free or united in elongated filaments. Water forms, not easily cultivable. Life energy derived mainly from oxidative

[4] These first three orders are included briefly to give the complete setting of the fourth, the Eubacteriales, with which we are primarily concerned.

processes. Cells typically containing either granules of free sulphur or bacterio-purpurin or both, usually growing best in the presence of hydrogen sulphide.

C. ORDER CHLAMYDOBACTERIALES[4]

Cells normally united in elongated filaments. Sulphur and bacterio-purpurin are absent. Iron often present and usually a well-marked sheath.

D. ORDER EUBACTERIALES. Ordo nov.

Synonyms: *Bacterina* Perty 1852 in part; *Eubacteria* Schroeter 1886; *Eubacteriaceae* A. J. Smith 1902.

The order Eubacteriales includes the forms usually termed the true bacteria, that is, those forms which are considered least differentiated and least specialized. The cell metabolism is not primarily bound up with hydrogen sulphide or other sulfur compounds, the cells in consequence containing neither sulfur granules nor bacterio-purpurin. The cells apparently do not possess a well-organized or well-differentiated nucleus. The cells are usually minute and spherical, rod-shaped or spiral in shape, in most genera not producing true filaments; the filaments when formed not sheathed, and frequently branching, thus being differentiated from the iron bacteria. The cells may occur singly, in chains or other groupings. The cells may be motile by means of flagella, or non-motile; they are never *notably* flexuous. Cell multiplication occurs always by transverse, never by longitudinal fission. Some genera produce endospores, particularly the rod-shaped types. More or less branching of cells and filaments may occur, reaching its maximum expression in the genera *Nocardia* and *Actinomyces* which may show typical mycelium formation, intergrading with the molds. Chlorophyll is absent, though the cells may be pigmented. The cells may be united into gelatinous masses, but never form motile pseudoplasmodia nor develop a highly specialized cyst-producing fruiting stage, such as is characteristic of the *Myxobacteriales*.

FAMILY I. NITROBACTERIACEAE Fam. nov.

Organisms usually rod-shaped (sometimes spherical in *Nitrosomonas* and possibly in *Azotobacter*). Cells motile or non-motile; when motile with polar, never peritrichous, flagella. Endospores never formed. Obligate aerobes, capable of securing growth energy by the direct oxidation of carbon, hydrogen or nitrogen or of simple compounds of these. Non-parasitic (usually water or earth forms).

1. *Hydrogenomonas* Jensen 1909

Monotrichic short rods capable of growing in the absence of organic matter, and securing growth energy by the oxidation of hydrogen (forming water). Kaserer (1905) who first described the organism states that his species will also grow well on a variety of organic substances.

The type species is *Hydrogenomonas pantotropha* (Kaserer) Jensen. Nikleuski (1910) described two additional species, *H. vitrea* and *H. flava*.

2. *Methanomonas* Jensen 1909

Monotrichic short rods capable of growing in the absence of organic matter and securing growth energy by the oxidation of methane (forming carbon dioxide an dhydrogen). The type species is *Methanomonas methanica* (Söhngen) Jensen.

3. *Carboxydomonas* Jensen 1909

Autotrophic rod-shaped cells capable of securing growth energy by the oxidation of carbon monoxide (forming carbon dioxide). The type species, *Carboxydomonas oligocarbophila* (Beijerinck and van Delden) Jensen, is described as non-motile.

4. *Mycoderma* Persoon 1822 emended

Synonyms: Ulvina Kuetzing 1837; *Umbina* Naegeli 1849; *Bacteriopsis?* Trevisan 1885; *Gliacoccus?* Maggi 1886; *Acetobacter* Fuhrmann 1905; *Acetimonas* Jensen 1909.

Cells rod-shaped, frequently in chains, non-motile. Cells grow usually on the surface of alcoholic solutions, securing growth energy by the oxidation of alcohol to acetic acid. Also capable of utilizing certain other carbonaceous compounds, as sugar and acetic acid. Elongated, filamentous, club-shaped, swollen and even branched cells common and quite characteristic.

The type species is *Mycoderma aceti* Thompson?

5. *Nitrosomonas* Winogradsky 1892

Includes *Nitrosococcus* Winogradsky 1892.

Cells rod-shaped or spherical, motile or non-motile, if motile with polar flagella. Capable of securing growth energy by the oxidation of ammonia to nitrites. Growth on media containing organic substances scanty or absent.

The type species is *Nitrosomonas europoea* Winogradsky.

6. *Nitrobacter* Winogradsky? 1892

Synonym: Nitrosobacterium? Rullmann 1897.

Cells rod-shaped, non-motile, not growing readily on organic media or in the presence of ammonia. Cells capable of securing growth energy by the oxidation of nitrites to nitrates.

Winogradsky named no species, although he described one. It might be termed ***Nitrobacter Winogradskyi*** and made the type species.

7. *Azotobacter* Beijerinck 1901

Synonyms: Parachromatium Beijerinck 1903; *Azotomonas* Jensen 1909.

Relatively large rods, or even cocci, sometimes almost yeast-like in appearance, dependent primarily for growth energy upon the oxidation of carbohydrates. Motile or non-motile; when motile, with tuft of polar flagella. Obligate aerobes usually growing in a film upon the surface of the culture medium. Capable of fixing atmospheric nitrogen when grown in solutions

containing carbohydrates and deficient in combined nitrogen. The best-known free-living nitrogen-fixing bacteria of the soil.

The type species is *Azotobacter chroöcoccum* Beijerinck.

8. *Rhizobium* Frank 1889

Synonyms: Phytomyxa Schroeter 1886; *Cladochytrium* Vuillemin 1888; *Rhizobacterium* Kirchner 1895; *Pseudorhizobium* Hartleb 1900; *Rhizomonas* Jensen 1909.

Comment. Phytomyxa Schroeter has priority over *Rhizobium*, but because of the confusion which would arise from the substitution of the older correct name for the better known term *Rhizobium*, the committee recommends the adoption of the latter.

Minute rods, motile when young by means of polar flagella. Involution forms abundant and characteristic when grown under suitable conditions. Obligate aerobes, capable of fixing atmospheric nitrogen when grown in the presence of carbohydrates in the absence of compounds of nitrogen. Produce nodules upon the roots of leguminous plants.

The type species is *Rhizobium leguminosarum* Frank.

FAMILY II. MYCOBACTERIACEAE Chester 1897

Cells usually elongated, frequently filamentous and with a decided tendency to the development of branches, in some genera giving rise to the formation of a definite branched mycelium. Cells frequently show swellings, clubbed or irregular shapes. Endospores not produced, but conidia developed in some genera. Usually Gram-positive. Non-motile. Many species are parasitic in animals or plants. Complex proteins usually required. As a rule strongly aerobic, (except for some species of *Actinomyces* and the genera *Fusiformis* and *Leptotrichia*), and oxidative. Growth on culture media often slow; some genera show mold-like colonies.

1. *Actinomyces* Harz 1877

Synonyms: Streptothrix Cohn 1875, not *Streptothrix* Corda 1839; *Discomyces* Rivolta and Micellone 1878; *Micromyces* Gruber 1891, not

Micromyces Dangeard 1888; *Oöspora* Sauvageau and Radais 1892; not *Oöspora* Wallroth 1833; *Cohnistreptothrix* Pinoy 1913.

Organism growing in form of a much-branched mycelium, which may break up into segments that function as conidia. Usually parasitic, with clubbed ends of radiating threads conspicuous in lesions in animal body. No *aerial* hyphae or conidia. Some species are microaerophilic or anaerobic. Non-motile.

The type species is *Actinomyces bovis* Harz.

2. *Nocardia* Trevisan 1889

Synonyms: Actinomyces of many authors; *Streptothrix* of many authors; *Thermoactinomyces* Tsilinsky 1899.

Branched filaments, resembling a mycelium, readily breaking up into segments, usually saprophytic soil forms. Differs primarily from *Actinomyces* in the development of *aerial* hyphae and conidia. Usually aerobic. Many are pigment formers. Colonies as a rule mold-like on culture media.

3. *Mycobacterium* Lehmann and Neumann 1896

Synonyms: Sclerothrix Metschnikoff 1888, not *Sclerothrix* Kuetzing 1849; *Coccothrix* Lutz 1886; *Mycomonas* Jensen 1909.

Slender rods which are stained with difficulty, but when once stained are acid-fast. Cells sometimes show swollen, clavate or cuneate forms, and occasionally even branched filaments. Non-motile, Gram-positive. No endospores. Growth on media slow. Aerobic. Several species pathogenic to animals.

The type species is *Mycobacterium tuberculosis* (Koch) Lehmann and Neumann.

4. *Corynebacterium* Lehmann and Neumann 1896

Synonyms: Corynemonas Jensen 1909; *Corynethrix* Bingert 1901.

Slender, often slightly curved, rods with tendency to club formation, branching cells occasionally seen in old cultures. Barred irregular staining. Not acid-fast. Gram-positive. Non-

motile. Aerobic. No endospores. Some pathogenic species produce a powerful exotoxin. Characteristic snapping motion is exhibited when cells divide.

The type species is *Corynebacterium diphtheriae* (Loeffler) Lehmann and Neumann.

5. *Fusiformis* Hoelling 1910

Synonym: Mantegazzaea Vuillemin 1913, not *Mantegazzaea* Trevisan 1879.

Obligate parasites. Cells usually elongate and fusiform, staining somewhat irregularly. Filaments sometimes formed; non-branching. Non-motile. No spores. Growth in laboratory media feeble.

The type species (?) is *Fusiformis termitidis* Hoelling.

6. *Leptotrichia* Trevisan 1879 emended

Synonyms: Leptothrix Robin 1847, not *Leptothrix* Kuetzing 1843; *Rasmussenia* Trevisan 1889.

Thick, long, straight or curved threads, frequently clubbed at one end and tapering to the other. Gram-positive when young. Threads fragment into short, thick rods. Anaerobic or facultative. Non-motile. Filaments sometimes granular; non-branching. No aerial hyphae or conidia. Parasites or facultative parasites.

The type species is *Leptotrichia buccalis* (Robin) Trevisan.

FAMILY III. PSEUDOMONADACEAE

Short rods, usually motile. Flagella single, polar. Gram-negative. Not obligate aerobes. Many species active ammonifiers. Many species produce water-soluble pigments or green fluorescence; yellow pigment common. Some species are photogenic. Soil and water bacteria, with many plant parasites.

1. *Pseudomonas* Migula 1894

Synonyms: Bactrillum Fischer 1895; *Arthrobactrimium* Fischer 1895; *Arthrobactrillum* Fischer 1895; *Eupseudomonas* Migula 1895; *Bactrinius* Kendall 1902; *Bactrillius* Kendall 1902; *Bacterium* Ehrenberg emended E. F. Smith 1905; *Denitromonas* Jensen 1909; *Liquidomonas* Jensen 1909.

Rod-shaped, short, usually motile by means of polar flagella or rarely non-motile. Aerobic and facultative. Frequently gelatin liquefiers and active ammonifiers. No endospores. Gram stain variable, though usually negative. Fermentation of carbohydrates as a rule not active. Frequently producing a water-soluble pigment which diffuses through the medium as green, blue, purple, brown, etc. In some cases a non-diffusible yellow pigment is formed. Many yellow species are plant parasites.

FAMILY IV. SPIRILLACEAE Migula 1894

Cells elongate, more or less spirally curved. Cell division always transverse, never longitudinal. Cells non-flexuous. Usually without endospores. As a rule motile by means of polar flagella, sometimes non-motile. Typically water forms, though some species are intestinal parasites.

1. *Vibrio* Miller 1773 emended E. F. Smith 1905

Synonyms: Pacinia Trevisan 1885; *Microspira* Schroeter 1886; *Pseudospira* DeToni and Trevisan 1889; *Liquidovibrio* Jensen 1909; *Solidovibrio* Jensen 1909; *Photobacterium?* Beijerinck 1889.

Cells short bent rods, rigid, single or united into spirals. Motile by means of a single (rarely two or three) polar flagellum, which is usually relatively short. Many species liquefy gelatin and are active ammonifiers. Aerobic and facultative. No endospores. Usually Gram-negative. Water forms, a few parasites.

The type species is *Vibrio cholerae* (Koch) Buchner.

2. *Spirillum* Ehrenberg 1830 emended Migula 1894

Synonyms: Spirobacillus? Metschnikoff 1889; *Spirosoma* Migula 1894; *Sporospirillum?* Jensen 1909.

Cells rigid rods of various thicknesses, length, and pitch of the spiral, forming either long screws or portions of a turn. Cells motile by means of a tuft of polar flagella (5 to 20) which are mostly half circular, rarely wavy-bent. These flagella occur on one or both poles; their number varies greatly and is difficult to determine, since in stained preparations several are often united into a common strand. Endospore formation has been reported in some species. Habitat; water or putrid infusions.

FAMILY V. COCCACEAE Zopf 1884 emended Migula 1894

Synonyms: Sphaerobacteria Cohn 1872; *Coccogenae* Trevisan 1885; *Coccacei* Schroeter 1886; *Coccobacteria* Schroeter 1886.

Cells in their free conditions, spherical; during division somewhat elliptical. Division in one, two or three planes. If the cells remain in contact after division they are frequently flattened in the plane of division. Motility rare. Endospores absent. Metabolism complex, usually involving the utilization of amino-acids or carbohydrates.

Tribe 1. Streptococceae Trevisan

Parasites (thriving only or best on or in the animal body) Grow well under anaerobic conditions. Many forms grow with difficulty on media, none very abundantly. Planes of fission usually parallel, producing pairs or short or long chains, never packets. Generally stain by Gram. Produce acid but no gas in glucose and lactose broth. Pigment, if any, white or orange.

1. *Neisseria* Trevisan 1885

Synonyms: Diplococcus Weichselbaum 1886 in part; *Gonoccoccus?* Neisser? 1879; *Merismopedia* Zopf 1885, not *Merismopedia* Meyen 1839.

Strict parasites, failing to grow or growing very poorly on artificial media. Cells normally in pairs of flattened cells. Gram-negative. Fermentative powers low. Growth fairly abundant on serum media, usually whitish or yellowish.

The type species is *Neisseria gonorrhoeae* Trevisan.

2. *Streptococcus* Rosenbach 1884 emended Winslow and Rogers 1905

Synonyms: Sphaerococcus Marpmann 1885, not *Sphaerococcus* Agardh 1821; *Perroncitoa* Trevisan 1889; *Babesia?* Trevisan 1889; *Schuetzia* Trevisan 1889; *Lactococcus* Beijerinck 1901; *Hypnococcus* Bettencourt et al. 1904; *Myxokokkus* Gonnermann 1907, not *Myxococcus* Thaxter 1892; *Melococcus?* Amiradzibi 1907; *Diplostreptococcus* Lingelsheim 1912.

Chiefly parasites. Cells normally in short or long chains (under unfavorable conditions, sometimes in pairs and small groups, never in large packets). Generally stain by Gram. Capsules and zooglea often formed. On agar streak, effused translucent growth, often with isolated colonies. In stab culture, little surface growth. Sugars fermented with formation of large amount of acid. Generally fail to liquefy gelatin or reduce nitrates.

Type species is *Streptococcus pyogenes* Rosenbach.

3. *Staphylococcus* Rosenbach 1884

Synonyms: Micrococcus Cohn 1872 em. Migula 1894; *Botryomyces* Bollinger 1888; *Botryococcus* Kitt 1888, not *Botryococcus* Kuetzing 1849; *Galactococcus* Guillebeau; *Bollingera* Trevisan 1889; *Gaffkya* Trevisan 1885; *Pyococcus* Ludwig 1892; *Carphococcus* Hohl 1902; *Aurococcus* Winslow and Rogers 1906, *Indolococcus* Jensen 1909; *Liquidococcus* Jensen 1909; *Peptonococcus* Jensen 1909; *Enterococcus?* (Thiercelin) Rougentzoff 1914.

Parasites. Cells in groups and short chains, very rarely in packets. Generally stain by Gram. On agar streak good growth, of orange color. Sugars fermented with formation of moderate amount of acid. Gelatin often liquefied very actively.

Type species is *Staphylococcus aureus* Rosenbach.

4. *Albococcus* Winslow and Rogers 1905

Differs from *Staphylococcus* in forming more abundant surface growth of porcelain white color, and in fact that liquefaction of gelatin when present is less vigorous.

Tribe 2. Micrococceae Trevisan

Facultative parasites or saprophytes. Thrive best under aerobic conditions. Grow well on artificial media, producing abundant surface growths. Planes of fission often at right angles; cell aggregates in groups, packets or zooglea masses. Generally decolorize by Gram. Pigment yellow or red.

5. *Micrococcus* Cohn 1872, emended Winslow and Rogers 1905

Synonyms: Microsphaera Cohn 1872, not *Microsphaera* Leveille 1851; *Pediococcus* Balcke 1884; *Merista* Van Tieghem 1884, not *Merista* (Banks and Soland) Cunningham 1839; *Planococcus* Migula 1894; *Urococcus* Miquel 1879, not *Urococcus* Kuetzing 1849; *Pedioplana* Wolff 1907; *Tetradiplococcus?* Bartoszewicz and Schwarzwasser 1908; *Solidococcus* Jensen 1909; *Planomerista* Vuillemin 1913.

Facultative parasites or saprophytes. Cells in plates or irregular masses (never in long chains or packets). Generally decolorize by Gram. Growth on agar abundant, with formation of yellow pigment. Glucose broth slightly acid, lactose broth generally neutral. Gelatin frequently liquefied, but not rapidly.

The type species is *Micrococcus luteus* (Schroeter) Cohn.

6. *Sarcina* Goodsir 1842, emended Winslow and Rogers 1905

Synonyms: Urosarcina Miquel 1879; *Planosarcina* Migula 1894; *Lactosarcina* Beijerinck 1908; *Sporosarcina?* Jensen 1909.

Sarcina differs from *Micrococcus* solely in fact that cell division occurs under favorable conditions in three planes, forming regular packets.

The type species is *Sarcina ventriculi* Goodsir.

7. *Rhodococcus* Flügge. 1891, emended Winslow and Rogers 1906

Synonyms: Not *Rhodococcus* Molisch 1907.

Saprophytes. Cells in groups or regular packets. Generally decolorize by Gram. Growth on agar abundant with formation of red pigment. Glucose broth slightly acid, lactose broth neutral. Gelatin rarely liquefied. Nitrates generally reduced to nitrites.

FAMILY VI. BACTERIACEAE Cohn 1872 emended

Rod-shaped cells without endospores. Gram-negative. Flagella when present peritrichic. Metabolism complex, amino-acids being utilized, and generally carbohydrates.

1. *Bacterium* Ehrenberg 1838, emended Jensen 1909

Synonyms: Actinobacter Duclaux 1882 in part; *Klebsiella* Trevisan 1885 in part; *Gliscrobacterium* Malerba and Sanna Salaris 1888; *Aerobacter* Beijerinck 1900; *Salmonella* Ligniéres 1900; *Denitrobacterium* Jensen 1909.

Motile or non-motile rods, staining evenly. Easily cultivable. Animal pathogens or saprophytes. Often chromogenic. Many forms actively decompose carbohydrates.

The type species is *Bacterium coli* Escherich.

2. ***Erwinia*** nov. gen.

Plant pathogens. Growth usually whitish, often slimy. Indol generally not produced. Acid usually formed in certain carbohydrate media, but as a rule no gas.

3. *Pasteurella* Trevisan 1887

Synonyms: Octopsis? Trevisan 1885; *Coccobacillus* Gamaleia 1888, not *Coccobacillus* Leube 1885.

Short rods, single or rarely in chains, usually showing distinct polar staining. Non-motile. Gram-negative. Without spores.

Aerobic and facultative. Powers of carbohydrate fermentation slight; no gas produced. Gelatin not liquefied. Parasitic, frequently pathogenic, producing plague in man and hemorrhagic septicemia in the lower animals.

The type species is *Pasteurella cholerae-gallinarum* (Flügge) Trevisan.

4. ***Hemophilus*** gen. nov.

Synonyms: Pyobacillus? Koppanyi 1907; *Diplobacillus* Morax 1896, not *Diplobacillus* Weichselbaum 1887.

Minute rod-shaped cells, non-motile, without spores, strict parasites, growing best (or only) in the presence of hemoglobin, and in general requiring blood serum or ascitic fluid. Gram-negative.

The type species is ***Hemophilus influenzae*** (Pfeiffer).

FAMILY VII. LACTOBACILLACEAE. Fam. nov.

Rods, often long and slender, Gram-positive, non-motile, without endospores. Usually produce acid from carbohydrates, as a rule lactic. When gas is formed, it is CO_2 without H_2. The organisms are usually somewhat thermophilic. As a rule microaerophilic; surface growth on media poor.

1. *Lactobacillus* Beijerinck 1901

Synonyms: Dispora? Kern 1882; *Saccharobacillus?* van Laer 1889; *Streptobacillus* Rest and Khoury 1902; *Brachybacterium* Troili-Petersson 1903; *Caseobacterium* Jensen 1909.

Generic characters those of the family.

The type species is *Lactobacillus caucasicus* (Kern?) Beijerinck.

FAMILY VIII. BACILLACEAE

Rods producing endospores, usually Gram-positive. Flagella when present peritrichic. Actively decompose protein media through the agency of enzymes.

1. *Bacillus* Cohn 1872

Synonyms: Bactrella? Morren 1830; *Metallacter?* Perty 1852; *Bactridium* Davaine 1868 in part; *Urobacillus* Miquel 1879; *Pollendera* Trevisan 1884; *Zopfiella* Trevisan 1885; *Streptobacter* Schroeter 1886; *Cornilia* Trevisan 1889 in part; *Bacterium* Ehrenberg, emended Migula 1894 in part; *Bactridium* Fischer 1895, not *Bactridium* Wallroth 1832; *Bactrinium* Fischer 1895; *Bactrillum* Fischer 1895; *Endobacterium* Lehmann and Neumann 1896; *Astasia* Meyer 1898; *Fenobacter* Beijerinck 1900; *Bacterius* Kendall 1902 in part; *Aplanobacter* E. F. Smith 1905 in part; *Semiclostridium* Maassen 1905; *Plennobakterium* Gonnermann 1907; *Myxobacillus* Gonnermann 1907; *Thermobacillus* Jensen 1909; *Serratia* Vuillemin 1913 in part, not *Serratia* Bizio 1823.

Aerobic forms. Mostly saprophytes. Liquefy gelatin. Often occur in long threads and form rhizoid colonies. Form of rod usually not greatly changed at sporulation.

The type species is *Bacillus subtilis* Cohn.

2. *Clostridium* Prazmowski 1880

Synonyms: Amylobacter Trecul 1865; *Cornilia* Trevisan 1889 in part; *Granulobacter* Beijerinck 1893; *Clostrillum* Fischer 1895; *Clostrinium* Fischer 1895; *Paracloster* Fischer 1895; *Semiclostridium* Maassen 1905; *Botulobacillus* Jensen 1909; *Butyribacillus* Jensen 1909; *Cellulobacillus* Jensen 1909; *Putribacillus* Jensen 1909.

Anaerobes. Often parasitic. Rods frequently enlarged at sporulation, producing clostridium or plectridium forms.

The type species is *Clostridium butyricum* Prazmowski.

ORGANISMS INTERMEDIATE BETWEEN BACTERIA AND PROTOZOA

SPIROCHAETACEAE Swellengrebel 1907

Free living or parasitic spirilliform organisms with or without flagella, with undulating or rigid spiral twists. Reproduction by transverse division and by "coccoid bodies," the equivalent of spores.

Four genera are recognized as follows:

1. *Spirochaeta* Ehrenberg. Non-parasitic, with flexible undulating body and with or without flagelliform tapering ends. Common in sewage and foul waters.

The type species is *Spirochaeta plicatilis* Ehrenberg.

2. *Cristispira* Gross. Giant forms with undulating body and peculiar flattened ridge erroneously called an "undulating membrane" which runs the length of the body. Parasitic in molluscs.

The type species is *Cristispira balbianii* Certes, from the crystalline style of the oyster.

3. *Saprospira* Gross. Non-parasitic forms similar to Cristispira, but without the flattened ridge or "crista" which is, if present, here replaced by a straight columella or thickening of the periplast.

The type species is *Saprospira grandis* Gross.

4. *Treponema* Schaudinn. Parasitic and frequently pathogenic forms with undulating or rigid spirilliform body. Without crista or columella. With or without flagelliform tapering ends.

The type species is *Treponema pallidum* Schaudinn.

1. ARTIFICIAL KEY TO THE ORDERS OF THE SCHIZOMYCETES

Cells united during the vegetative stage into a pseudoplasmodium ..... A. Myxobacteriales

Cells not forming a pseudoplasmodium

Cells free or united in elongated filaments, often with a well-defined sheath. Conidia frequently formed. Free sulphur, iron or bacbacterio-purpurin often present.

Cells typically containing granules of sulphur or bacterio-purpurin or both ..... B. Thiobacteriales

Sulphur and bacterio-purpurin absent; iron often present ..... C. Chlamydobacteriales

Cells never in sheathed filaments. Conidia only in the mycelial Mycobacteriaceae. Flagella often present. Free iron, sulphur, or bacterio-purpurin never present ..... D. Eubacteriales

2. ARTIFICIAL KEY TO THE FAMILIES OF THE EUBACTERIALES

Cells spiral with polar flagella ..... IV. Spirillaceae

Not as above

Cells spherical; rarely, if ever, motile; spores never produced; never securing growth energy from nitrogen or ammonia ..... V. Coccaceae

Not as above
Cells short rod-shaped with a single rarely two polar flagellum; usually forming green or yellow pigment.....III. PSEUDOMONADACEAE
Not wholly as above
Spores formed.....VIII. BACILLACEAE
Spores never formed
Metabolism simple, securing growth energy from carbon, hydrogen, or their simple compounds; flagella, if present, polar
I. NITROBACTERIACEAE
Metabolism complex, dependent upon more complex carbohydrate and protein substances; flagella, if present, peritrichic
Cells clubbed, fusiform, filamentous, branching or mycelial; those not distinctly so are either acid-fast or show barred irregular staining.....II. MYCOBACTERIACEAE
Not as above
Gram-positive; non-motile.....VII. LACTOBACILLACEAE
Gram-negative; often motile.....VI. BACTERIACEAE

3. ARTIFICIAL KEY TO THE GENERA OF THE EUBACTERIALES

I. NITROBACTERIACEAE
Fixing nitrogen or oxidizing its compounds
Fixing nitrogen
Cells large; in soil.....7. *Azotobacter*
Rods minute; in roots of leguminous plants.....8. *Rhizobium*
Oxidizing nitrogen compounds
Oxidizing ammonia.....5. *Nitrosomonas*
Oxidizing nitrites.....6. *Nitrobacter*
Not as above
Oxidizing hydrogen.....1. *Hydrogenomonas*
Oxidizing carbon compounds
Oxidizing alcohol; branching forms common.....4. *Mycoderma*
Not as above, using simpler carbon compounds
Oxidizing CO.....3. *Carboxydomonas*
Oxidizing CH_4.....2. *Methanomonas*
II. MYCOBACTERIACEAE
Slender rods, staining with difficulty and acid-fast....3. *Mycobacterium*
Not as above
Mycelium and conidia formed
With aerial hyphae and conidia; usually saprophytic soil organisms.....2. *Nocardia*
Hyphae and conidia not aerial; usually parasitic in animals
1. *Actinomyces*
Not as above; cells rod-like, usually somewhat curved, clubbed, fusiform, or even branched, but never mycelial
Thick, long threads, fragmenting into short thick rods
6. *Leptotrichia*

Not as above
Cells usually elongate and fusiform; filaments, if formed, not branching; staining somewhat irregularly.....5. *Fusiformis*
Cells slightly curved, clubbed, or in old cultures even branching; not filamentous; showing definitely barred staining.....4. *Corynebacterium*

III. PSEUDOMONADACEAE
Generic characters mainly those of family.....1. *Pseudomonas*

IV. SPIRILLACEAE
Flagellum single (rarely 2 or 3).....1. *Vibrio*
Flagella tufted (5–20).....2. *Spirillum*

V. COCCACEAE
Abundant red-pigmented growth on agar.....7. *Rhodococcus*
Not as above
Gram-negative
Normally in pairs of flattened cells; growth on plain agar scanty, never bright yellow.....1. *Neisseria*
Normally in plates, packets, or irregular masses; growth on plain agar abundant, pigment definitely yellow
Cells in regular packets.....6. *Sarcina*
Cells not in regular packets.....5. *Micrococcus*
Gram-positive (Exceptions rare and not easily confused with above genera)
Cells normally in chains, sometimes in pairs (especially in acid environment) never in large irregular masses. Gelatine rarely liquefied. Growth on plain agar usually translucent, never heavy, never yellow or orange.....2. *Streptococcus*
Cells normally in groups or masses; (occasionally in plates in Albococcus?) chains short and irregular, if present. Gelatine often liquefied. Agar growth abundant, white to orange
Pigment orange (rarely lacking); gelatine often liquefied actively.....3. *Staphylococcus*
Whitish to porcelain white; liquefaction less vigorous 4. *Albococcus*

VI. BACTERIACEAE
Plant pathogens.....2. *Erwinia*
Not as above; saprophytes or in animal habitats (intestines, tissues, etc.)
Usually motile and exhibiting active fermentative powers; typically parasitic in intestines of man and higher animals; growing well on ordinary media.....1. *Bacterium*
Not wholly as above
Growing only in presence of hemoglobin, ascitic fluid or serum 4. *Hemophilus*
Growth on media scanty, but less sensitive than the above; short rods with tendency to bipolar stain.....3. *Pasteurella*

VII. LACTOBACILLACEAE
Generic characters mainly those of family.....1. *Lactobacillus*
VIII. BACILLACEAE
Aerobic, usually saprophytic; cells not greatly enlarged (if at all) at sporulation.....1. *Bacillus*
Anaerobic, often saprophytic; cells frequently enlarged at sporulation.....2. *Clostridium*

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COUNTING THE LIVING BACTERIA IN MILK—A PRACTICAL TEST

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The method under test, in the series of analyses reported here, has already been described (1915, 1916a). A comparison of results obtained in a series of milk analyses by this and the standard plate method has also appeared (1916 b). The following advantages have been claimed for the method:

1. It is rapid, requiring only about one-eighth of the time needed for the standard plate method.

2. The technic is simple and requires little glassware or culture medium.

3. It furnishes a means of keeping a permanent record of each analysis.

4. Only the individual bacteria, or groups of the same, that are alive grow into colonies. This makes it possible to use the method for counting the bacteria in pasteurized milk, which is not possible by methods of direct microscopical examination such as those of Slack and Breed.

It seemed desirable to make further tests of the method, and if possible, under practical conditions, such as working in connection with the routine examination of a city milk supply. Through the kindness of Dr. M. J. Rosenau of the Department of Preventive Medicine and Hygiene of the Harvard Medical School, arrangements were made for me to carry on a series of tests in the laboratories of the Boston Board of Health. The work has been made possible further by the courtesy of Dr. F. H. Slack, director of the laboratories, and Dr. W. M. Campbell, who conducts the bacteriological work on milk.

In this laboratory all samples of milk collected by the milk

TABLE 1

The number of bacteria per cubic centimeters of milk obtained by different methods

DATE	NO.	KIND OF MILK	MICROSCOPICAL ESTIMATE (BLACK)	STANDARD PLATES				LITTLE PLATES (FROST)	RATIO
				Routine test		Duplicate (Frost)			
				Number of colonies	Bacteria per cubic centimeter	Number of colonies	Bacteria per cubic centimeter	Bacteria per cubic centimeter	
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
I. March 15, 1916	(1) 1	Pasteurized	−	14–10	120,000			52,000	1 : 0.43
	(2) 2	Pasteurized		7–10	85,000			28,600	1 : 0.34
	(3) 3	Raw	+	253–228	2,500,000			1,560,000	1 : 0.62
	(4) 4	Pasteurized	−	23–29	200,000			197,600	1 : 0.98
	(5) 5	Pasteurized	−	9–17	130,000			31,200	1 : 0.24
	(7) 6	Pasteurized	−	13–15	140,000			54,000	1 : 0.38
	(8) 7	Pasteurized	−	24–25	245,000			218,400	1 : 0.88
	(9) 8	Pasteurized	−	116–155	1,400,000			1,040,000	1 : 0.74
	(10) 9	Pasteurized	++	70–31	500,000			1,560,000	1 : 3.1
	(11) 10	Pasteurized	+	237–204	2,000,000			1,690,000	1 : 0.84
	(12) 11	Pasteurized	+	223–193	2,000,000			854,000	1 : 0.43
II. March 16, 1916	(1) 12	Pasteurized		164–139	135,000			59,150	1 : 0.43
	(2) 13	Pasteurized	+	29–sp.	290,000			96,200	1 : 0.33
	(3) 14	Pasteurized	−	63–79	710,000			2,080,000	1 : 2.90
	(4) 15	Pasteurized	−	24–22	230,000			187,200	1 : 0.81
	(5) 16	Pasteurized	−	20–22	210,000			65,000	1 : 0.31
	(6) 17	Pasteurized	−	136–128	132,000			13,000	1 : 0.10
	(7) 18	Pasteurized	−	62–76	690,000			1,040,000	1 : 1.50
	(8) 19	Pasteurized	−	54–39	460,000			130,000	1 : 0.28
	(9) 20	Pasteurized	+	29–26	270,000			640,000	1 : 2.40
	(10) 21	Pasteurized	−	98–102	100,000			85,800	1 : 0.85
	(11) 22	Pasteurized	+	8–6	7,000,000			1,560,000	1 : 0.22
	(12) 23	Pasteurized	+	9–sp.	1,900,000			780,000	1 : 0.41

NOTE − = microscopic estimate below 500,000. + = estimate of 500,000 and over. Sp. = spreaders. Column 5 gives counts on duplicate plates.

III. March 17, 1916	(1)	24	Raw	−	Spreaders				182,000	
	(2)	25	Raw	−	Spreaders				132,000	
	(3)	26	Pasteurized	−	12–18	150,000			49,400	1 : 0.33
	(4)	27	Pasteurized?	+	68–74	700,000			1,040,000	1 : 1.46
	(5)	28	Pasteurized	−	20–10	150,000			7,800	1 : 0.05
	(6)	29	Pasteurized	−						
	(8)	30	Pasteurized	−	25–15	200,000			200,000	1 : 1
	(9)	31	Pasteurized	−	10–4	70,000			78,000	1 : 1.11
	(10)	32	Pasteurized?	−	4–16	100,000			260,000	1 : 2.60
	(11)	33	Pasteurized	−	34–48	500,000			936,000	1 : 1.87
	(12)	34	Pasteurized	−	12–4	80,000			78,000	1 : 0.97
IV. March 18, 1916	(1)	35	Raw	−	Spreaders				41,600	
	(2)	36	Raw	−	Spreaders				62,400	
	(3)	37	Raw	−	Spreaders				44,200	
	(4)	38	Raw	−	Spreaders				18,200	
	(5)	39	Pasteurized	−	8–0	40,000			3,120,000	1 : 78
	(6)	40	Pasteurized?	+	178–184	1,800,000			1,716,000	1 : 0.95
	(7)	41	Pasteurized	−	6–18	120,000			640,000	1 : 5.33
	(8)	42	Pasteurized	−	7–sp.	70,000			210,000	1 : 3.
	(9)	43	Pasteurized	−	10–18	140,000			1,040,000	1 : 7.43
	(10)	44	Pasteurized	+	133–127	1,200,000			13,780,000	1 : 11.50
	(11)	45	Raw	−	Spreaders				338,000	
				−	Spreaders				200,000	
V. March 20, 1916	(1)	46	Pasteurized	−	Spreaders				10,400	
	(2)	47	Pasteurized	−	Spreaders				18,200	
	(3)	48	Pasteurized	−	Spreaders				117,000	
	(4)	49	Pasteurized	−	Spreaders				832,000	
	(5)	50	Pasteurized	−	Spreaders				182,000	
	(6)	51	Pasteurized	−	Spreaders				8,800	
	(7)	52	Pasteurized	−	Spreaders					
	(8)	53	Pasteurized	−	6–2	40,000			1,157,000	1 : 29
	(9)	54	Pasteurized	+	64–80	700,000			1,339,000	1 : 1.91
	(10)	55		+	Spreaders					
	(11)	56		+	338–363	3,500,000			1,300,000	1 : 0.37
	(12)	57		−	8–6	70,000			43,900	1 : 0.62

TABLE 1—*Continued*.

DATE	NO.	KIND OF MILK	MICROSCOPICAL ESTIMATE (SLACK)	STANDARD PLATES				LITTLE PLATES (FROST)	RATIO
				Routine test		Duplicate (Frost)			
				Number of colonies	Bacteria per cubic centimeter	Number of colonies	Bacteria per cubic centimeter	Bacteria per cubic centimeter	
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
VI. March 21, 1916	(1) 58	Raw	—	13–14	120,000	3–2	30,000	33,800	1 : 0.25 : 0.28
	(2) 59	Raw	—	0–0	−10,000	1–2	10,000	5,200	1 : 1 : 0.52
	(3) 60	Raw	—	10–6	80,000	11–12	110,000	20,800	1 : 1.25 : 0.25
	(4) 61	Raw	—	7–5	60,000	10–0	50,000	26,000	1 : 80 : 0.45
	(5) 62	Raw	—	11–9	90,000	sp–5	50,000	39,000	1 : 0.55 : 0.43
	(6) 63	Raw	—	0–0	−10,000	6–sp	60,000	7,800	1 : 6 : 0.78
	(7) 64	Raw	—	1–3	20,000	1–2	10,000	7,800	1 : 0.50 : 0.39
	(8) 65	Raw	—	0–0	−10,000	2–sp	20,000	3,250	1 : 2 : 0.32
	(9) 66	Raw	—	0–0	−10,000	1–4	25,000	ap.13,000	1 : 2.5 : 1.30
	(10) 67	Raw	—	5–3	40,000	5–2	35,000	11,830	1 : 0.9 : 0.30
	(11) 68	Raw	—	5–1	30,000	2–2	20,000	27,400	1 : 0.66 : 0.90
	(12) 69	Raw	—	0–0	−10,000	13–3	80,000	22,750	1 : 8 : 2.27
VII. March 22, 1916	(1) 70	Pasteurized	—	6–2	40,000	sp–5	50,000	26,000	1 : 1.25 : 0.65
	(2) 71	Pasteurized	—	10–16	130,000	15–14	150,000	114,000	1 : 1.15 : 0.80
	(3) 72	Pasteurized	—	37–41	400,000	36–sp	360,000	520,000	1 : 0.90 : 1.30
	(4) 73	Pasteurized	—	10–8	90,000	−5–7	50,000	41,600	1 : 0.55 : 0.45
	(5) 74	Pasteurized	—	3–15	90,000	37–28	320,000	267,000	1 : 3.55 : 3.00
	(6) 75	Pasteurized	—	14–16	150,000	10–13	120,000	197,000	1 : 0.80 : 1.31
	(7) 76	Pasteurized	—	25–3	140,000	22–sp	220,000	44,200	1 : 1.57 : 0.31
	(8) 77	Pasteurized	—	sp–3	30,000	3–3	30,000	15,000	1 : 1 : 0.50
	(9) 78	Pasteurized	—	11–5	80,000	9–10	100,000	35,750	1 : 1.25 : 0.44
	(10) 79	Pasteurized	—	48–53	500,000	160–120	1,400,000	1,222,000	1 : 2.80 : 2.44
	(11) 80	Pasteurized	—	29–31	300,000	24–20	200,000	299,000	1 : 0.66 : 1.00

VIII. March 23, 1916	(1)	81	Raw	+	246–257	2,500,000	sp–340	3,400,000	1,500,000	1 : 1.36 : 0.60
	(2)	82	Pasteurized	−	10–12	110,000	1–2	10,000	260,000	1 : 0.09 : 2.30
	(3)	83	Pasteurized	−	37–43	400,000	27–sp	270,000	520,000	1 : 0.67 : 1.30
	(4)	84	Pasteurized	−	27–24	250,000	30–sp	300,000	20,000	
	(5)	85	Pasteurized	+	49–56	500,000	sp–8–8	80,000	1,000,000	1 : 0.14 : 2
	(6)	86	Pasteurized	−	11–sp	110,000	10–sp	100,000	104,000	1 : 0.90 : 0.95
	(7)	87	Pasteurized	−	10–2	60,000	11–sp	110,000	20,000	
	(8)	88	Pasteurized	−			10–11	100,000	100,000	1 : 1
	(9)	89	Pasteurized	−	10–20	150,000	11–25	180,000	30,000	
	(10)	90	Pasteurized	−	19–23	200,000	10–10	100,000	36,800	1 : 0.50 : 0.18
	(11)	91	Pasteurized	−	39–sp	400,000	54–45	500,000	653,000	1 : 1.25 : 1.63
	(12)	92	Cream	+	0–0		sp–0		no growth	
IX. March 24, 1916	(1)	93	Raw	+	1–0	60,000	1–0	10,000	26,000	1 : 0.16 : 0.43
	(2)	94	Raw	+	6–sp	60,000	0–3	15,000	12,000	1 : 0.25 : 0.20
	(3)	95	Raw	+	6–sp	60,000	sp–2	10,000	13,000	1 : 0.16 : 0.21
	(4)	96	Raw	+	sp–mold		33–36	350,000	91,000	
	(5)	97	Raw	+	0–0	−10,000	4–1	25,000	30,000	1 : 2.50 : 3.00
	(6)	98	Raw	+	74	750,000	40–20	300,000	300,000	1 : 0.40 : 0.40
	(7)	99	Raw	+	3–4	40,000	6–3	45,000	104,000	1 : 1.12 : 2.60
	(8)	100	Raw	+	8–sp	70,000	sp9–sp5	70,000	73,000	1 : 1.00 : 1.05
	(9)	101	Raw	+	0–0	−10,000	0-0sp	−10,000	very few	
	(10)	102	Raw	+	400–800	6,000,000	368–268	3,120,000	1,040,000	1 : 0.48 : 0.16
X. March 27, 1916	(21)	103	Pasteurized	−	184–sp	1,800,000	sp–sp		+1,000,000	
	(3)	104	Pasteurized	−	146–sp	1,460,000	520–sp	520,000	5,200,000	1 : 0.35 : 3.5
	(9)	105	Pasteurized	−	24–sp	250,000	9–4	650,000	26,000	1 : 2.60 : 0.1
	(20)	106	Pasteurized	+	24	24,000,000	sp–sp		2,600,000	
	(25)	107	Pasteurized	−	sp	500,000	sp–sp		3,000,000	
	(27)	118	Pasteurized	−	19	190,000	sp–sp			
	(28)	109		−	24	250,000	sp–sp		1,560,000	
	(29)	110		−	67	650,000	sp–sp		1,830,000	
	(30)	111		−	88	900,000	230–sp	2,300,000	3,120,000	1 : 2.5 : 3.4

inspector and sent into the laboratory are examined microscopically by the Slack method, and rated as above or below the legal limit of 500,000 bacteria per cubic centimeter. Those samples rated as likely to show more than the permissible number of bacteria are plated out, as also are all samples of pasteurized milk. The presence of streptococci and pus is noted. The microscopical estimate is used further as a guide to the dilution needed in plating. Ordinarily duplicate plates of only one dilution are made. This is usually 1: 10,000.

At the time the routine plates were poured I made little plates. From sample 59 to sample 111 (table 1) I also made standard plate cultures.

The medium used for the ordinary plates was made according to the standard methods. It had a reaction of +1.5 and contained one per cent of agar. Ten cubic centimeters were used for each plate. The Petri dishes had clay tops. The temperature of incubation was 37.5°, and the time forty-eight hours. The air of the incubator was saturated with moisture. The counting was done either with the naked eye or with the aid of a reading glass.

The little plates were made in the manner previously described (Frost, 1916 a) and incubated five to seven hours. They were then dried, stained and counted. The same agar was used as that supplied the laboratory. It would have been better, no doubt, to have had a medium containing more agar, since some difficulty was experienced from spreaders, and in highly contaminated milks the little colonies were not so well individualized as would have been the case with a stiffer agar.

The results obtained from these comparative tests are given in table 1. The kind of milk is indicated, i.e., whether raw or pasteurized. The microscopical estimate by the Slack method; the plate counts obtained in the routine analyses, together with the number of colonies actually seen on the plates; the plate counts obtained by the writer; the results obtained from the use of the little plates; and the ratio which the results obtained by the different methods of analysis bear to each other, are indicated in the different columns of the table.

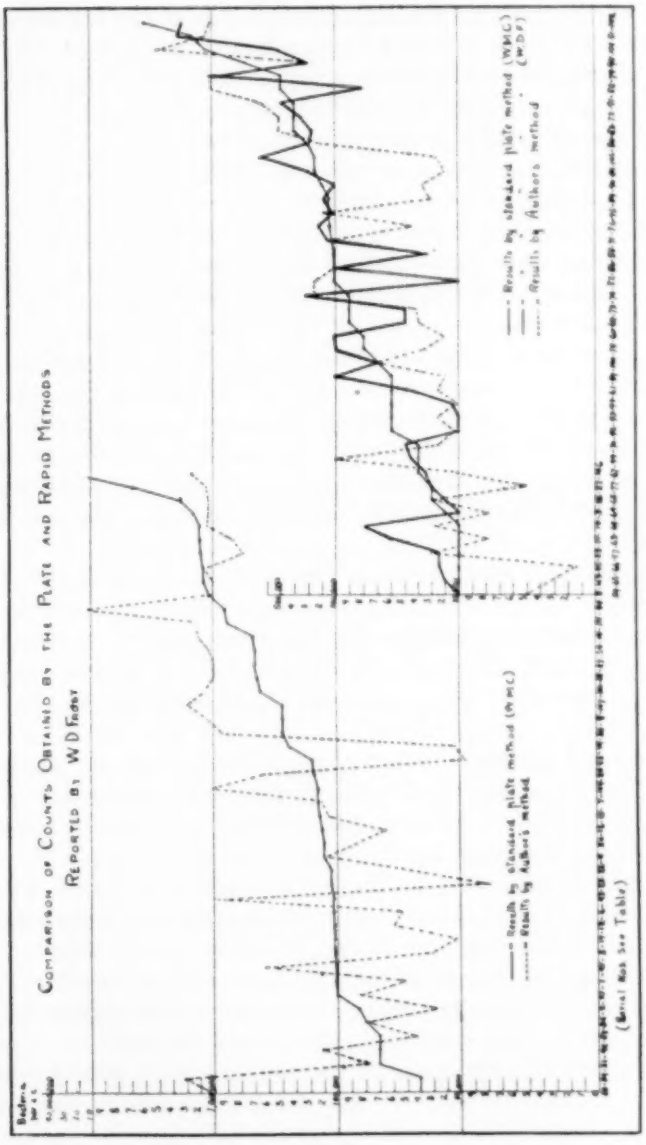
Comparison of Counts Obtained by the Plate and Rapid Methods
Reported by W D Frost
Results by standard plate method (WWC)
Results by Authors method
Results by standard plate method (WWC)
Results by Authors method (WDF)

CHART 1

All of the analyses made during the time of the comparative tests are included in the table, although it is apparent that the data are too unsatisfactory in a number of cases to be of much value; yet for the sake of completeness all are given.

The general results are shown in chart 1.

The milks used were both raw and pasteurized, and varied in bacterial content from very good to highly contaminated milks.

One of the first things to note is the fact that because of spreaders on both plates (which unfortunately were unusually prevalent in the laboratory at the time of these tests), no counts could be obtained in 16 out of the 111 tests, and in 16 other samples one of the duplicate plates was spoiled by spreaders.

While spreaders occurred on the little plates under exactly the same conditions, their presence did not materially interfere with a count of the plates, with the possible exception of one. It would seem then that the little plates could be relied upon to give countable colonies with more certainty than the standard plates.

The second thing to notice is that there is considerable variation between the counts on the duplicate plates of the routine series. In ten samples the difference amounted to over 50 per cent (5, 9, 26, 28, 30, 43, 71, 78, 89, and 102) and in eleven other samples it was over 100 per cent (31, 32, 34, 39, 41, 64, 68, 70, 74, 76, and 87).

Again, the number of colonies on the plates in about 40 cases was below 20, which would seem to be the smallest number of colonies that can be relied upon as giving anything like a satisfactory degree of accuracy.

Excluding those samples in which one-half or more of the plates were rendered useless because of spreaders, and also those samples in which both plates had less than twenty colonies, it will be seen that only 28 of the 111 counts can be considered as thoroughly reliable in indicating the exact number of bacteria in the sample when the standard plate method was used.

The criticisms here made of the routine plates would apply with equal force to the duplicate Petri dish cultures made by myself. Of 53 only 10 were entirely satisfactory in that both

plates were free from spreaders and had a sufficient number of colonies present to be thoroughly reliable.

The agreement between the two series of standard plates varies from a ratio of 1:0.09 to 1: 8, with an average ratio of 1:1.42. In other words, the plates that I made from one sample contained slightly less than one-tenth as many colonies (9 per cent) as the routine plates, and in another instance my count was 700 per cent higher than the routine, while my average figures were 42 per cent higher than those of Dr. Campbell.

The little plates, on the other hand, were all countable. Some of them were too thickly seeded, and others were overrun to some extent with spreaders, but neither of these things prevented the counting of the plates. It might have been expected, for several reasons, that the counts obtained by this method would not closely approximate the count obtained by the standard plate method, the chief of which is that the medium is somewhat different from the standard in that it is half milk. Again, the short period of incubation might not be sufficient for the development of colonies from some of the slowly-growing bacteria; and finally, crowding might be sufficient to inhibit the growth of some colonies. In spite of these differences and possible handicaps, however, the results obtained in this competitive series show a reasonable agreement.

Compared with the routine standard plates (leaving out of account three of the samples [39, 44 and 53] which are evidently not comparable), the ratio varied from 1:0.05 to 1:7.43, with an average of 1:0.96. This appears to be a better showing than was obtained in duplicate runs by the standard plate method alone where the average variation was 1:1.42. It must be pointed out, however, that this comparison gives a false impression, since the counts obtained by the little plates are on the whole somewhat lower than those obtained by the standard plate method. For example, if we compare the results I obtained by the standard plate method with the routine plates made by Dr. Campbell, we will see that my count fell below the routine count nineteen times, while it was higher in 14 samples. That is, the variation occurred either up or down with about

equal frequency. On the other hand, comparing the results obtained from the little plates with the results from the routine plates, it is found that in 48 samples the count on the little plates fell below the count obtained by the standard plate method, and above in 26 samples. In other words, the count on the little plates was lower than that on the standard plates in two cases, as against one above.

There are marked differences between the results obtained by the two methods in several samples. Some figures on the little plates are very much lower than those obtained by the standard plate method. This might be accounted for by the supposition that colonies did not have time to develop in the short period of incubation, but in some of these cases, at least, a long period of incubation had little effect in raising the count. What seems more likely in these instances is that we are dealing with samples of milks in which the bacteria have a tendency to form groups

*Tests comparing the plating of 120 cc. of whole milk with the ordinary dilution methods.** (*By W. M. Campbell*)

1/20 cc. WHOLE MILK		DILUTION 1:20		DILUTION 1:100	
Number of colonies	Count	Number of colonies	Count	Number of colonies	Count
712	14,240	1,109	22,180	401	40,100
688	13,760	1,042	20,840	365	36,500
181	3,620	257	5,140	98	9,800
173	3,460	229	4,580	87	8,700
660	13,200	870	17,400	220	22,000
720	14,400	680	13,600	200	20,000
185	3,700	415	8,300	141	14,100
616	12,320	1,030	20,600	331	33,100
202	4,040	210	4,200	49	4,900
200	4,000	237	4,740	128	12,800
232	4,640	129	2,580	71	7,100
340	6,800			224	22,400
449	8,980			217	21,700
128	2,560	200	4,000		
110	2,200	240	4,800		
118	2,360	128	2,560		
96	1,920	140	2,800		

* American Journal Public Hygiene, **17**, p. 359.

that are not easily shaken apart except as they are diluted with water. This fact has been brought out especially well by a series of tests given in the Reports of the Boston Board of Health (1907).

This grouping of the bacteria no doubt occurs to some extent in all milks, but appears to be more noticeable in the better grades of milk.

The little plates occasionally give much higher counts than the standard plate method; see, for example, nos. 39, 44, and 53 (table 1). In these samples the little plates showed a large number of small colonies of the lactic acid type, and it is quite conceivable that the count obtained by the little plates is more accurate than that obtained by the standard plate method.

TABLE 2

Number of bacteria in plain and lactose agars—from Sherman 1916

SAMPLE NUMBER*	NUMBER OF BACTERIA PER CUBIC CENTIMETER		
	Plain agar	Lactose agar	Ratio
1	817	1,017	1:1.25
2	1,340	2,070	1:1.55
3	1,630	2,000	1:1.23
4	3,230	3,530	1:1.09
(3)	6,000	69,000	1:10.50
(2)	6,500	42,500	1:6.54
5	7,330	7,330	1:1
(1)	7,600	25,300	1:3.33
6	8,000	11,300	1:1.41
7	8,900	12,100	1:1.36
8	9,030	8,970	1:0.99
9	11,800	11,170	1:0.95
(4)	18,100	43,600	1:2.41
(5)	23,800	58,000	1:2.44
(6)	72,000	177,000	1:2.46
10	186,000	610,000	1:3.28
11	192,000	278,000	1:1.45
12	260,000	361,000	1:1.39
13	369,000	463,000	1:1.26
14	53,700,000	109,000,000	1:2.03

* The numbers of the samples are the same as those used by Sherman in his table 1. The numbers in parenthesis are from his table 2. The last four analyses (nos. 15–18) are not given, because of the difficulty of charting such high numbers. See chart 2.

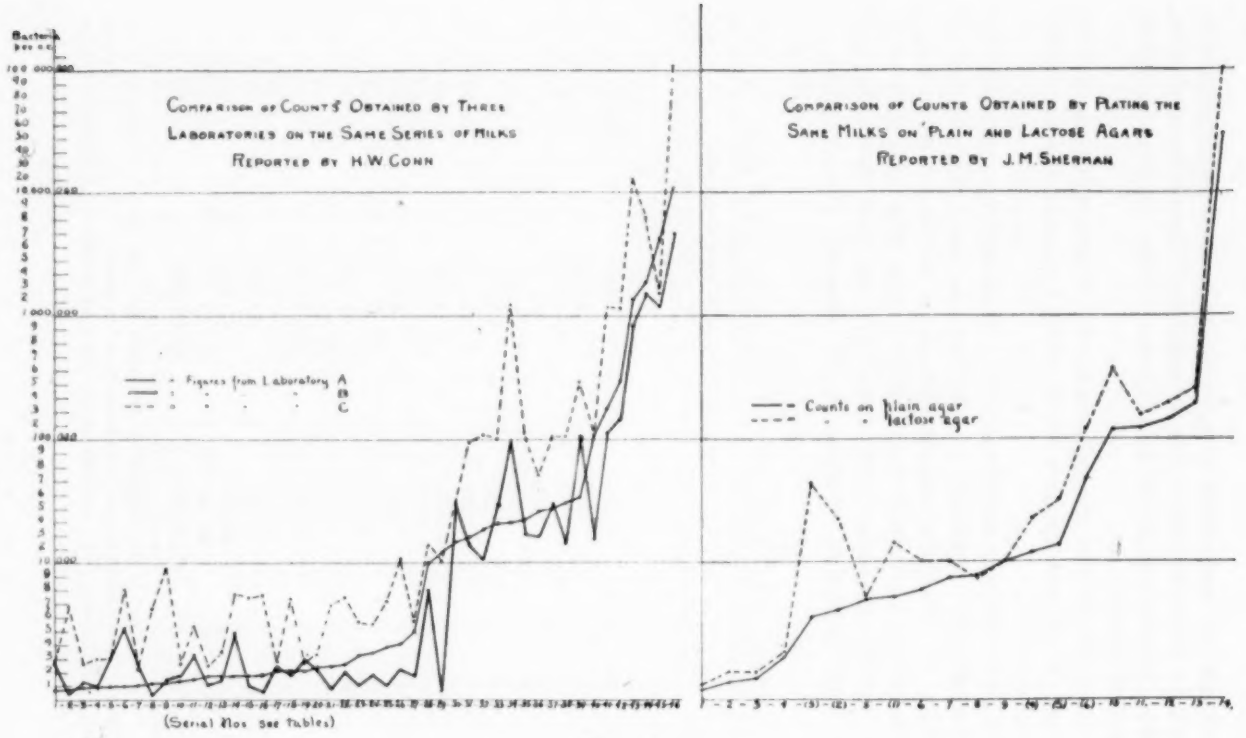
Comparison of Counts Obtained by Three Laboratories on the Same Series of Milks Reported by H.W. Conn
Figures from Laboratory A B C
(Serial Nos. see tables)
Comparison of Counts Obtained by Plating the Same Milks on Plain and Lactose Agars Reported by J.M. Sherman
Counts on Plain agar
Lactose agar

CHART 2

Sherman (1916) has recently shown that sugar agar gives higher counts than plain agar on the same milks. I have combined his tables 1 and 2 in my table 2. These figures are represented graphically in chart 2.

The large amount of milk in the little plates makes this a sugar medium, and it is possible that the discrepancies under discussion can be at least partially explained on this basis.

As far as the accuracy of the little plate method is concerned, when judged by the standard plate method, this study would seem to indicate that on the average the results are in fairly close agreement, with the probability that in about half of the samples the count will be somewhat low, and that other samples will show a much higher count than that obtained in the usual way.

It is interesting, in this connection, to compare the results obtained by different workers using the standard plate method in analyzing milk, and for this purpose I have brought together some of the results obtained by Conn (1915), and incorporated them in table 3. The figures were obtained by taking alternate numbers in Conn's table 2 and arranging them in an ascending scale. The column headed "S. Method and S. Media" was taken, but any other would apparently have given similar variations. The same figures are represented in chart 2.

TABLE 3

Data from "Standards for Determining the Purity of Milk". H. W. Conn. Table 3, p. 2366, Public Health Reports, August 13, 1915. Averages obtained by laboratories A. B. and C. Rearranged in one series using alternate figures.

RESULTS OBTAINED FROM THE SAME MILKS BY			RATIO
Laboratory A	Laboratory B	Laboratory C	
760	2,960	2,725	1:3.9:3.5
800	610	6,900	1:0.7:8.6
950	1,350	2,780	1:1.4:2.9
1,000	1,010	3,000	1:1.0:3.0
1,000	3,100	3,000	1:3.1:3.0
1,010	5,330	8,130	1:5.2:8.1
1,150	2,770	2,250	1:2.4:2.0
1,200	500	6,700	1:0.4:5.6
1,250	1,586	9,650	1:1.3:7.7
1,340	1,840	2,760	1:1.3:2.0

TABLE 3—*Continued*

RESULTS OBTAINED FROM THE SAME MILKS BY			RATIO
Laboratory A	Laboratory B	Laboratory C	
1,500	3,310	5,381	1:2.2:3.6
1,810	1,160	2,500	1:0.6:1.3
1,830	1,530	3,500	1:0.8:1.9
1,830	5,500	7,800	1:3.0:4.2
1,840	1,100	7,450	1:0.6:4.0
1,850	700	7,710	1:0.3:4.1
2,050	2,560	2,880	1:1.2:1.4
2,100	1,975	7,375	1:0.9:3.5
2,130	2,960	2,725	1:1.3:1.2
2,440	2,320	3,560	1:0.9:1.4
2,660	900	6,930	1:0.3:2.6
2,700	2,175	7,580	1:0.8:2.8
3,325	1,200	5,750	1:0.3:1.7
3,450	2,950	5,510	1:0.7:1.6
3,900	2,100	7,210	1:0.5:1.8
4,100	2,230	14,300	1:0.5:3.5
5,050	1,975	5,750	1:0.4:1.1
11,560	8,000	24,000	1:0.7:2.1
17,200	760	12,300	1:0.04:0.6
25,100	57,000	55,000	1:2.2:2.1
29,000	22,500	99,000	1:0.7:3.4
35,000	13,300	138,000	1:0.3:4.0
39,200	53,000	113,000	1:1.3:3.0
40,000	198,000	1,730,000	1:4.9:43.0
42,300	30,500	145,000	1:0.7:3.4
49,000	29,000	75,000	1:0.5:1.5
52,600	55,000	121,000	1:1.4:2.3
54,600	24,500	124,000	1:0.4:2.2
59,100	135,000	523,000	1:2.2:8.8
130,000	27,400	146,000	1:0.2:1.1
337,000	158,000	1,700,000	1:0.3:5.0
533,000	244,000	1,600,000	1:0.4:3.0
2,350,000	930,000	22,000,000	1:0.4:9.0
3,500,000	2,700,000	8,190,000	1:0.8:2.4
6,720,000	1,600,000	2,700,000	1:0.2:0.4
14,600,000	7,000,000	164,000,000	1:0.5:11.2

The variations, it will be noticed, are similar to those found in comparing my method with the standard plate method, and I believe I am warranted in saying that variations very similar in magnitude to those obtained by using the standard plate method and the little plate method may occur when two or more workers analyze milk by the standard plate method alone.

In considering the accuracy of this method, one of the things that must be determined is the uniformity of the distribution of the colonies on the little plates. Experience in these tests is that the distribution of colonies in the various fields in any little plate is quite uniform. To show this, the counts of twenty fields are given here by the mechanical selection of these fields on ten different plates.

NO. 1 LOW	NO. 5 LOW	NO. 10 OIL IM.	NO. 15 OIL IM.	NO. 20 LOW	NO. 30 LOW	NO. 40 OIL IM.	NO. 50 LOW	NO. 60 LOW	NO. 70 LOW
13	10	8	13	42	62	8	62	0	10
7	9	11	13	53	50	7	62	1	6
6	10	10	12	41	55	11	58	3	12
16	9	6	15	40	57	12	58	2	7
10	8	3	8	34	68	17	62	2	7
10	10	4	8	24	54	19	66	2	8
7	11	3	8	24	47	20	62	0	8
17	13	10	5	26	44	9	46	2	6
16	11	9	5	22	50	9	43	2	8
14	14	11	6	27	58	17	38	3	13
18	13	6	6	25	39	11	43	4	11
11	21	4	6	45	40	13	34	4	7
10	15	3	3	35	50	13	57	1	9
15	9	7	9	29	43	13	53	0	12
16	14	9	5	33	54	13	59	1	11
10	14	7	8	26	60	10	56	4	9
11	8	6	11	32	50	11	55	3	6
6	13	6	12	25	39	14	70	2	4
10	14	4	8	22	53	9	68	2	9
10	19	3	6	35	47	12	68	1	12
20)233	20)245	20)130	20)167	20)640	20)1020	20)248	20)1120	20)39	20)175
12	12	6.5	8	32	51	12	56	2	9

Another matter of importance is counting at different magnifications. In a former communication (1916 b) some emphasis

was laid on the discrepancies obtained with different magnifications used for counting the little plates. The data presented were for counts where the period of incubation was only four or five hours. If the period of incubation is seven or eight hours there seems to be little difference, and that magnification may be used which will give the most satisfactory number to count.

In comparing the two methods other considerations need brief mention.

First, the method is rapid, requiring not over seven or eight hours.

Second, the little plate method is reliable in that it is not likely to fail entirely because of spreaders, too great a dilution, etc.

Third, it permits a study of a relatively large amount of the milk, usually 1/20th of a cubic centimeter.

Fourth, it furnishes a permanent record of the bacterial content of the milk.

Fifth, it requires less material in the way of glassware and media. Microscopical slides, sterilized in the flame just before use, are substituted for Petri dishes, and the amount of medium is not over 1/20th and may be as little as 1/200th part of that used by the standard plate method.

Sixth, it is less time-consuming to make the little plates than it is to make the standard plates, and the only previous preparation necessary is the sterilization of pipettes and test tubes for mixing the samples. The counting of the little plates requires more time if 20 fields are carefully gone over. It seems probable, however, that the extensive counting may not be required in routine work. If the little plate is gone over quickly to gain an idea of the distribution of the colonies, the counting of four or five representative fields will be sufficient. Tests of this kind made during the progress of this work gave very satisfactory results. By varying the magnification, fields with comparatively few colonies in them can always be obtained.

The method may seem complicated to some, but in reality it is not, and if one will take the time to master the principles involved, the method is much simpler than the standard plate method.

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STUDIES ON THE BACTERIAL METABOLISM OF SULFUR

I. FORMATION OF HYDROGEN SULFIDE FROM CERTAIN SULFUR COMPOUNDS UNDER AEROBIC CONDITIONS

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Hydrogen sulfide is a decomposition product of many sulfur-containing compounds, and its formation by bacteria from both organic and inorganic substances has received much attention. An interesting paper on this subject is that of Sasaki and Otsuka, (1912). These investigators used Fränkel's protein-free medium as the substrate to which were added cystine, neutral sulfur, taurine, sodium thiosulfate, sodium sulfite, and sodium sulfate. All of the 21 pure cultures which they used formed hydrogen sulfide from cystine, with the exception of *Ps. pyocyaneus* and *Ps. fluorescens*. The pyogenic cocci were able to reduce neutral sulfur only. Burger (1914) observed hydrogen sulfide formation from cystine by a few common bacteria, but none of their strains produced it from taurine, peptone or sodium thiosulfate. Incubation periods of 12, 24 and 48 hours were used.

EXPERIMENTAL

In the course of some work that was being done with the fluorescent group of bacteria the action of these organisms, together with other strains of common bacteria, was tested upon cystine and other sulfur-containing compounds. The fluorescent bacteria used in this investigation were isolated from water. The other forms were secured from the American Museum of

Natural History, with the exception of the Bacillus of winter cholera.[1]

Fränkel's solution was used for the substrate. This was made up according to the following formula.

Sodium chloride	5 grams
Monocalcium phosphate	2 grams
Ammonium lactate	6 grams
Asparagin	4 grams
Distilled water	1000 cc.
$\frac{N}{1}$ NaOH	20 cc.

After the various sulfur compounds had been added the media were placed in test tubes and sterilized in the Arnold steam sterilizer. Inoculations were made with small portions of growth from a young slant agar culture.

To detect the formation of hydrogen sulfide, strips of bibulous paper which had been saturated with lead acetate solution containing glycerol were suspended in the top of each culture tube. The tubes were then plugged with cotton and incubated at 25°C. for thirty days, at the end of which time observations were made for darkening of the lead acetate paper. A full set of control tubes was employed. In no instance did the control tubes darken the indicator paper, which points to the fact that the hydrogen sulfide, when present in the culture tubes, came from the sulfur compounds in the substrate.

PEPTONE

Fränkel's medium with 4 per cent added peptone was used. Sixty-two of the 97 fluorescent bacteria formed hydrogen sulfide from peptone. Four of these strains produced it from peptone and not from cystine. This may be taken as evidence of other sulfur linkings in protein than that of cystine. Practically all of the pure cultures were able to split hydrogen sulfide from peptone.

[1] This organism was received from Mr. A. J. Hinkelmann, Galesburg, Illinois, and is described in the Illinois Medical Journal, November, 1915.

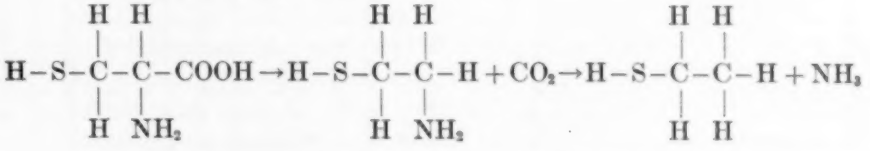
CYSTINE

The cystine used in this experiment was prepared from wool, according to Folin's method (Folin, 1910) and purified by repeated recrystallizations.[2] It was used in the strength of 6 per cent in Fränkel's medium. On account of the small supply of cystine, small culture tubes holding about 5 cc. were employed.

After incubation for thirty days 86 of the 97 strains of fluorescent bacteria had formed hydrogen sulfide from cystine. At the same time 34 strains of other common bacteria were inoculated into this medium, most of which formed hydrogen sulfide. The results are indicated in the table. *B. paratyphi "B," B. enteritidis,* the Bacillus of winter cholera, and *B. coli* formed large amounts of hydrogen sulfide from cystine in much less time than 30 days.

These data differ from those reported by Sasaki and Otsuka (1912). The fluorescent bacteria in their investigation formed no hydrogen sulfide. The discrepancy between the results of Sasaki and Otsuka and those reported in this paper may be due to the period of incubation. The former adopted one week as the incubation period, while in this investigation it seemed best to lengthen the period to 30 days. Many of the fluorescent bacteria isolated from water produced no hydrogen sulfide until after the fifteenth day. Most of the intestinal bacteria produced it in less than a week.

The decomposition of cystine has been studied from a chemical and biochemical viewpoint, but very few definite data are available. Mathews (1915) believes that in digestion in the intestine cystine is first formed, and that this later undergoes decarboxylation and deaminization to yield ethyl mercaptan, according to the following equation.



[2] A portion of this was secured from Dr. H. B. Lewis of the division of physiological chemistry. The rest was prepared directly from wool.

The above changes are thought to be probable on account of the presence of mercaptans in the feces. Neuberg and Asher (1907), upon the dry distillation of cystine, obtained amino-ethyl-disulfide, $S_2(CH_2-CH_2-NH_2)_2$. It is improbable that this

TABLE 1

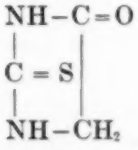
NAME OF BACTERIUM	NUMBER OF STRAINS	HYDROGEN SULFIDE FORMATION IN															
		Peptone		Cystine		2 thiohydantoin		Thiourea		Taurine		$Na_2S_2O_3$		$MgSO_4$		Na_2SO_3	
		+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−
Fluorescent bacteria	97	62	35	85	12		10*	62	35		97	42	55		97		97
B. coli	2	2	−	2	−	0	0	−	2	−	2	2	−	−	2	−	2
B. paracoli	1	1	−	1	−	0	0	1	−	−	1	1	−	−	1	−	1
B. enteritidis	1	1	−	1	−	−	1	1	−	−	1	1	−	−	1	−	1
B. alkaligines	1	1	−	1	−	0	0	1	−	−	1	−	1	−	1	−	1
B. cloacae	1	1	−	1	−	0	0	1	−	−	1	1	−	+	1	−	1
B. aerogenes	1	1	−	1	−	0	0	−	1	−	1	1	−	−	1	−	1
B. pyogenes-fecalis	1	1	−	1	−	0	0	−	1	−	1	1	−	−	1	−	1
B. paratyphi A	1	1	−	−	1	0	0	1	−	−	1	−	1	−	1	−	1
B. paratyphi B	1	1	−	1	−	−	1	−	1	−	1	1	−	−	1	−	1
B. typhi	1	1	−	1	−	0	0	−	1	−	1	−	1	−	1	−	1
B. capsulatus	3	3	−	3	−	0	0	1	2	−	1	3	−	−	3	−	3
Staph. albus	1	1	−	−	1	0	0	−	1	−	1	−	1	−	1	−	1
Staph. citreus	1	−	1	−	1	0	0	−	1	−	1	−	1	−	1	−	1
M. ureae	1	1	−	1	−	0	0	1	−	−	1	1	−	−	1	−	1
B. proteus	1	1	−	1	−	0	0	1	−	−	1	1	−	−	1	−	1
Clam bacillus	1	1	−	−	1	0	0	−	1	−	1	−	1	−	1	−	1
Sarcina ventriculi	1	−	1	−	1	0	0	−	1	−	1	−	1	−	1	−	1
B. butyricus	1	1	−	−	1	0	0	−	1	−	1	−	1	−	1	−	1
Bacillus of winter cholera	1	1	−	1	−	0	0	1	−	−	1	1	−	−	1	−	1
B. subtilis	1	−	1	−	1	0	0	−	1	−	1	−	1	−	1	−	1
Ps. pyocyaneus	1	−	1	+	−	0	0	−	1	−	1	−	1	−	1	−	1
M. tetragenus	1	−	1	−	1	0	0	1	−	−	1	−	1	−	1	−	1
B. cyaneus	1	1	−	−	1	0	0	−	1	−	1	−	1	−	1	−	1
Ps. phosphorescens	1	−	1	−	1	0	0	−	1	−	1	−	1	−	1	−	1
B. smegmae	1	1	−	−	1	0	0	−	1	−	1	−	1	−	1	−	1
B. arborescens	1	1	−	−	1	0	0	−	1	−	1	−	1	−	1	−	1
B. cereus	1	1	−	1	−	0	0	−	1	−	1	−	1	−	1	−	1
B. granulosus	1	−	1	−	1	0	0	1	−	−	1	−	1	−	1	−	1
B. mesentericus	1	1	−	1	−	0	0	1	−	−	1	1	−	−	1	−	1
M. flavus	1	−	1	−	1	0	0	−	1	−	1	−	1	−	1	−	1
B. dysenteriae	1	1	−	1	−	0	0	−	1	−	1	1	−	−	1	−	1

* Only the first ten strains were used in a 2 thiohydantoin medium.

compound would be formed in cellular metabolism, because the sulfur in cystine seems to be split off with little difficulty. Wohlgemuth (1905) reported that cystine which had been fed to rabbits in the diet appeared as increased sulfates in the urine and as taurine in the bile. Some of the hydrogen sulfide which many investigators have found in the urine may have come from cystine as well as sulfates.

The question of sulfur linkages in protein has received much attention. Johnson (1911) has given a good resumé of this subject. Many investigators have produced evidence which indicated that the sulfur in protein may not be fully accounted for by the cystine linkage. Bacteria may have a selective action, and may be able to attack only the sulfur in protein which is loosely combined. Johnson has prepared some thioamides of amino acids which offer more possibilities for the linkage of sulfur in protein.

2-THIOHYDANTOIN



In this compound sulfur has replaced the oxygen of the hydantoin nucleus. It has been studied by Johnson (1911, 1912) and his co-workers. Lewis (1912) has shown that the hydantoin nucleus is excreted as such when introduced into the organism of the cat, rabbit, or dog. In a later paper (Lewis, 1913) the same author reports a study of the behavior of 2-thiohydantoin when injected subcutaneously into rabbits. It was found "that approximately 0.125 gram per kilo body weight is the lethal dose for 2-thiohydantoin, while amounts of over 1.5 grams of hydantoin have been fed to rabbits without any toxic effects. The difference in reaction toward the animal body seems to rest in the sulfur of the 2-thiohydantoin.

Through the kindness of Dr. H. B. Lewis, a small amount of

2-thiohydantoin was secured upon which to try the action of bacteria. This was prepared from ammonium thiocyanate and hippuric acid, as described by Johnson and Nicolet (1911). The 2-thiohydantoin was dissolved in sterile water to yield a saturated solution. One cubic centimeter of this was added to 5 cc. of Fränkel's solution. The small amount of this compound limited the experiment to a few bacteria. The results are indicated in the table.

None of the bacteria formed the slightest amount of hydrogen sulfide detectable by the method used. The color of the solution changed upon incubation from the reddish orange of a solution of 2-thiohydantoin to a pale yellow. This may be regarded as indirect evidence that the compound was in some way broken down. Growth in all cases took place with the formation of a thick pellicle and a heavy sediment. This lack of toxicity for bacteria is of interest, in view of the marked toxicity for higher forms of life already referred to.

THIOUREA

A 10 per cent solution of this compound in Fränkel's medium was used. Thirty-four of the 97 fluorescent strains formed no hydrogen sulfide from this agent. Results with the other bacteria which were used may be found in the table. The formation of hydrogen sulfide from thiourea is of some interest, since none of the strains formed hydrogen sulfide from 2-thiohydantoin, a compound having the same sulfur linking, bivalent sulfur replacing bivalent oxygen. In peptone and thiourea substrates the fluorescent bacteria are correlated with regard to the number of strains forming hydrogen sulfide. This apparent correlation is probably of no significance. A strain which reduced the sulfur in peptone to hydrogen sulfide often failed to show this ability with regard to thiourea.

TAURINE

This was prepared from desiccated ox-bile and purified by repeated recrystallization (Hawk, 1916). None of the bacteria

used in this investigation reduced taurine to hydrogen sulfide, as determined by the method here employed. These results agree with those of Sasaki and Otsuka.

SODIUM THIOSULFATE

A 0.3 per cent solution of this compound in Fränkel's medium was used. Fifty-seven of the 97 fluorescent bacteria reduced the sulfur in sodium thiosulfate to hydrogen sulfide. The amount was not always large, but sufficient to cause a distinct change in the color of the lead acetate paper. The results for the other bacteria are indicated in the table. Lederer (1913) secured the formation of hydrogen sulfide when sodium thiosulfate was used in the place of sodium sulfate. Similar results have been reported by others.

MAGNESIUM SULFATE

To determine this property, Fränkel's solution was not used, but one proposed by Sullivan (1905) as being especially favorable to the development of fluorescent bacteria. It has the following composition.

Asparagin	10 grams
Magnesium sulfate	2 grams
K_2HPO_4	1 gram
Distilled water	1000 cc.

None of the bacteria reduced magnesium sulfate to hydrogen sulfide. This is in accord with the work of Sasaki and Otsuka and that of Lederer, and is probably due to the aerobic methods of culturing which were used. Beijerinck (1895, 1896,—) has shown that oxygen must be absent in order to secure formation of hydrogen sulfide from sulfate. An organism isolated by Zelinski (1893) seems to be the only one to which has been attributed the ability to form hydrogen sulfide from sulfate under both aerobic and anaerobic conditions.

SODIUM SULFITE

A 3 per cent solution of this compound was used in Fränkel's medium. None of the strains employed in this study reduced the

compound. This may have been due to the fact that very little growth was secured in the medium. Two of the strains, however, gave good growth, but no hydrogen sulfide. Sodium sulfite is known to inhibit the enzymes of the intestinal tract, and it may have acted in the same way with regard to the bacteria.

SUMMARY

The ability to form hydrogen sulfide from peptone is a rather wide-spread characteristic of the bacteria used in this study, including the fluorescent bacteria. With regard to cystine, the ability to liberate sulfur was possessed by almost all of the organisms used. The fluorescent bacteria and a few other forms are able to split hydrogen sulfide from thiourea with apparent ease, but do not attack 2-thiohydantoin to yield hydrogen sulfide. No hydrogen sulfide was formed from taurine and magnesium sulfate, both of which contain sulfur in more highly oxidized forms. The results with taurine and magnesium sulfate are in accord with those of Sasaki and Otsuka, indicating that bacteria may be unable to reduce highly oxidized sulfur with the formation of hydrogen sulfide. The results with sodium thiosulfate differ slightly from those reported by other investigators. With regard to sodium sulfite, no strain reduced sulfur to hydrogen sulfide, but this may be due to the concentration used. More work on this subject is planned.

I wish to acknowledge my indebtedness to Dr. Howard B. Lewis for his personal interest and timely suggestions.

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